CODED AMINO ACIDS : CHEMICAL STUDIES ON SITE SPECIFIC SIDE CHAIN ALTERATION

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in Partial Fulfilment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

by

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to the

DEPARTMENT OF CHEMISTRY

INDIAN INSTITUTE OF TECHNOLOGY KANPUR

JULY, 1988

Dedicated

to

My Parents

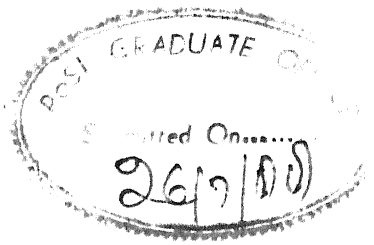
STATEMENT

I hereby declare that the matter embodied in this thesis is the result of investigations carried out by me at the Department of Chemistry, Indian Institute of Technology, Kanpur, India, under the supervision of Professor S. Ranganathan.

In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work embodied is based on the findings of other investigators.

D Bhattacharyya

(Dipti Bhattacharyya)



CERTIFICATE

Certified that the work contained in this thesis, entitled, "CODED AMINO ACIDS : CHEMICAL STUDIES ON SITE SPECIFIC SIDE CHAIN ALTERATION" has been carried out by Ms. Dipti Bhattacharyya under my supervision and the same has not been submitted elsewhere for a degree.

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CERTIFICATE OF COURSE WORK

This is to certify that Ms. Dipti Bhattacharyya has satisfactorily completed all the course requirements for the Ph.D. degree programme. The courses include :

Chm 502 Advanced Organic Chemistry

Chm 505 Principles of Organic Chemistry

Chm 524 Modern Physical Methods in Chemistry

Chm 525 Principles of Physical Chemistry

Chm 545 Principles of Inorganic Chemistry

Chm 581 Basic Biological Chemistry

Chm 612 Frontiers in Organic Chemistry

Chm 800 General Seminar

Chm 801 Graduate Seminar

Chm 900 Postgraduate Research

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To my husband, Rakesh, my indebtedness knows no bounds.

Dipti Bhattacharyya

PREFACE

The thesis entitled, "CODED AMINO ACIDS : CHEMICAL STUDIES ON SITE SPECIFIC SIDE CHAIN ALTERATION" consists of six parts, namely, A. Introduction, B. Background, C. Present Work, D. Spectra, E. Experimental and F. References.

SUMMARY OF THE PRESENT WORK

The work presented in this section endeavours to illustrate that chemoselective methodologies can be developed to effect side chain alterations in peptides, and that the results obtained in the present work could be transplanted to even more complex functional systems.

Chemical methodologies and strategies to effect enzyme alteration would necessarily entail less energy compared to the current methods where the starting point for such changes is at the genome level. The directed site specific protein alterations, requiring highly precise changes in an extremely complex molecular system built up with 20 coded amino acids, can be expected to be quite difficult. However, the advent of a vast array of strategies and tools available in the domain of organic chemistry has ushered in efforts towards effecting protein alteration using chemical methodologies.

The present work has endeavoured to develop a novel methodology pertaining to chemoselective protein modification. In order to achieve maximum versatility, two aspects were considered important, namely, that the transformation envisaged should lead to a product which, in turn, can be further elaborated

to several altered peptides (CHART S.1* = CHART C.IV), and, at the same time, the methodology should have a broad base in the sense that it should have the capability of affecting as many side chains of the 20 coded amino acids as possible. It was felt that such a broad base, by further developments could be further refined via various controls to achieve increasing selectivity, thus enabling the targeting to a selected residue amongst many which are also susceptible.

Of the 20 coded amino acids incorporated in the information system (CHART S.2 = CHART C.I), as many as 13, in principle, are susceptible to oxidizing agents (CHART S.3 = CHART C.V). Consequently, the oxidative methodology became the logical starting point for the amino acid side chain alteration studies. The 20 coded amino acids exhibit a range of oxidizing profiles, from the most difficult phenylalanine to the most readily oxidizable methionine. It was felt that the only reagent that could bring about the oxidation of such a range of substrates is the in situ generated Ru^{VIII} species. The present work, thus, is the account of the reaction of such amino acid side chains with in situ generated Ru^{VIII} species and culminates in the demonstration of possibilities for using this methodology for chemoselective protein modification.

The plan of research envisaged three stages of development, namely, the transformation of N,C-protected amino acid side chains, the transformation of amino acid side chains in dipeptides and higher peptides in competitive and non-competitive circumstances, and finally the demonstration of the methodologies thus developed in more complex proteins to effect chemoselective alteration.

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*In charts and tables, the notation "S" stands for "Synopsis" and "C" for the section outlining the present work (SECTION C) in the thesis.

In the present work, in addition, the action of Ru^{VIII} on unprotected amino acids has been investigated, in order to provide mechanistic support envisaged in the oxidative transformations, as well as to clarify conflicting reports present in the literature.

N-Benzyloxycarbonyl phenylalanine methyl ester, (1)*, on treatment with 2.2 mol% of Ru^{VIII} reagent in presence of $NaIO_4$ (18 mmol / mmol of substrate), employing H_2O-MeCN-CCl_4 as media at rt for 60 h gave ZAsp(β-OH)-OMe, (32), in 85% yields. The total lack of reactivity of the "Z" -protecting group in the above transformation towards Ru^{VIII} was confirmed by treatment of ZGlyOMe, (3), under conditions of the (1)→(32) change, resulting in its complete recovery. Even AcPheOMe, (2), underwent oxidative transformation to AcAsp(β -OH)OMe, (33), in 70% yields without affecting the N-acyl protecting unit (CHART S.4 = CHART C.XX).

N-Benzoyl tyrosine methyl ester, (4), similarly gave BzAsp(β-OH)-OMe, (34, 75%), even when the reaction time was reduced to 12 h, conditions under which (1) was hardly affected, thus indicating possible selectivity under competitive environments between the plenylalanine and the tyrosine side chains (CHART S.4 = CHART C.XX). The oxidation of either ZPheOMe or ZTyrOMe leading to ZAsp(β -OH)OMe is insensitive to the pH of the media, in the range 3-9. The transformation of ZPheOMe to ZAsp(β -OH)OMe represents a useful route to this rather difficultly preparable compound.

N-Benzoyl tryptophan methyl ester, (6), on oxidation with Ru^{VIII}, gave a 65% yield of BzAsp(β -OH)OMe and, in addition, N^{α}-benzoyl N^{ω}-formyl-

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*These numbers refer to those assigned in the present work (SECTION C and SECTION E).

kynurenine methyl ester, (35, 14%) and benzamide (13%). Similarly, N-benzyloxycarbonyl tryptophan methyl ester, (5), gave ZAsp(β-OH)OMe, (32, 65%) (CHART S.5 = CHART C.XXII).

The (6)→(34) change represents a novel oxidative degradation resulting in the loss of the entire benzenoid moiety.

Ancillary experiments have enabled the rationalization of this unusual change on the basis of four stages, three of which involve distinct types of oxidation by Ru^{VIII} (CHART S.6 = CHART C.XXV). Thus, compound (6) is oxidized initially to (35). Hydrolysis of (35) followed by oxidation leads to an α-keto acid which via oxidative degradation affords BzAsp(β-OH)OMe, (34). The intermediacy of N^{α}-Bz N^{ω}-ForKyn-OMe, (35), in the overall degradation of tryptophan to aspartic acid has been established via controlled oxidation of BzTrpOMe with only 2 eq. of periodate leading to, exclusively, (35), and the further transformation of (35) to BzAsp(β-OH)OMe, (34), in 97% yields under conditions of the (6)→(34) change. In view of the established inertness of the benzenoid moiety in (6) and other substrates towards the Ru^{VIII} species, (vide supra), it was considered reasonable to assume that the sensitive N-formamido grouping present in (35) most likely undergoes hydrolysis to N^{α}-benzoyl kynurenine methyl ester. This notion is supported by the transformation of tetrahydrocarbazole to adipic acid in 61% yields under conditions of the (6)→(34) change. The formation of adipic acid clearly establishes hydrolysis of the initially oxidized product since, if this were not to be the case, the product would have retained the aryl - N bond. The further oxidation of the o-adipoyl aniline thus formed can be expected to give rise to, by loss of the aromatic moiety, 2-keto pimelic acid, which, via oxidative decarboxylation, would lead

to adipic acid (CHART S.7 = CHART C.XXIV). The oxidative decarboxylation proposed as the last step in the (6)→(34) change was tested by attempting to effect a one step conversion of α-amino acids to lower carboxylic acids by oxidation, since, these substrates are unsually transformed first into α-keto acids. This was achieved. Valine was transformed into iso-butyric acid (66%) under the general oxidation conditions, and phenylalanine to phenylacetic acid (43%) in a shorter reaction time of 8 h (CHART S.8 = CHART C.XXVI). Thus, in the BzTrpOMe, (6) → N^{α}-Bz N^{ω}-ForKyn-OMe, (35) → N^{α}-BzKyn-OMe → N^{α}-BzGlu(γ-oxo)OMe → BzAsp(β-OH)OMe, (34) sequence of transformations, it so happens that the latent possibilities for the oxidation of the indole ring have found expression, leading to its conversion to a carboxyl group (CHART S.6 = CHART C.XXV).

The reaction of N-benzyloxycarbonyl histidine methyl ester, (7), with Ru^{VIII} species gave ZAsp(β-OH)OMe, (32, 25%) and the novel amino acid , N-benzyloxycarbonyl aspartoyl urea α-methyl ester, (36, 22%). The formation of compound (36) must involve the oxidation of the imidazole at the 2-location. Collapse of the resulting intermediate should lead to a highly electrophilic 2,4-bisazacyclopentadieneone system, which can be anticipated to undergo ready hydration to a vicinal diol. The latter, via unexceptional cleavage brought about by RuO_4, would result in the formation of N-benzyloxycarbonyl N^{ω}-formyl aspartoyl urea α-methyl ester. This common intermediate could lead, via two possible hydrolytic pathways, to either ZAsp(β-OH)OMe, (32), or ZAsp(β-$NHCONH_2$)OMe, (36) (CHART S.9 = CHART C.XXVIII). The main features envisaged in the above transformation, namely the acceptance of RuO_4 at the 2-location, collapse leading to the 2,4-bisazacyclopentadieneone system and its hydration leading to a vicinal diol finds support in the reaction of tetrahydro-

benzimidazole under conditions of the (7)→(32) + (36) change to 4,5-dihydroxy hexahydrobenzimidazole 2-one.

The oxidation of N-benzoyl methionine methyl ester, (8), with Ru^{VIII} yielded, surprisingly, BzMet(SO_2)OH, (38, 65%) and a mere 7% of the corresponding ester, (37). Blank experiments clearly demonstrated that a prior S-oxidation is necessary for the ester hydrolysis. The unusual transformation of (8) to (38) is rationalized on the basis of an intramolecular cyclization of the initially formed sulfoxide followed by hydrolysis of the resulting activated ester and further oxidation (CHART S.10 = CHART C.XXIX). The cyclic intermediate arising from interaction of the initially formed methionine S-oxide with the ester function can be expected to be highly reactive. It could undergo hydrolysis leading to the carboxylic acid (38), or, as was demonstrated in parallel studies with N-benzyloxycarbonyl methionine methyl ester, can undergo opening via intramolecular participation of the more basic benzyloxycarbonyl group. The incursion of such a reaction pathway was demonstrated on treatment of N-benzyloxycarbonyl methionine methyl ester, (9), which gave, in addition to the expected sulfone, (39, 40%), the unusual N-methyloxycarbonyl methionine sulfone methyl ester, (40, 18%). The formation of (40), in sum, involves the replacement of the benzyloxycarbonyl protecting group by a methoxycarbonyl unit and can be understood on the basis of alternate pathways, cited above, followed by hydrolysis, further oxidation, bicarbonate opening and diazomethane esterification (CHART S.11 = CHART C.XXX).

The oxidation of N-benzyloxycarbonyl S-benzyl cysteine methyl ester, (10), with Ru^{VIII} gave the sulfone ester, (41, 30%). The corresponding ZCys(SO_2-Bzl)-OH could not be detected in this oxidation which may be due

to difficulties associated with the realization of the transition state leading to the reactive intermediate arising from intramolecular cyclization of the initially formed sulfoxide (vide supra) (CHART S.12 = CHART C.XXXI).

The reaction of N-benzoyl proline methyl ester, (11), with Ru^{VIII} gave N-benzoyl pyroglutamic acid methyl ester, (42, 40%) and N-benzoyl glutamic acid α-methyl ester, (43, 17%) (CHART S.13 = CHART C.XXXII).

N-Benzoyl serine methyl ester, (12), exhibited a dual reaction profile with Ru^{VIII}. No reaction was observed at pH 3. However, in water, under the usual conditions of oxidation, the reaction led to the formation of benzamide (58%). In contrast, the oxidation of N-benzoyl threonine methyl ester, (13), with Ru^{VIII} at pH 3 gave a 84% yield of benzamide. A similar transformation also took place at pH 6 affording benzamide in lower yields (25%). The formation of benzamide in the reactions of (12) and (13) can be rationalized on the basis of a Ru-complex incorporating the vicinal amino-alcohol unit followed by fragmentation and hydrolysis. This reaction, therefore, has potential for application for peptide rupture at serine and threonine sites(CHART S.14=CHART C.XXXIV).

N-Benzyloxycarbonyl glutamine methyl ester, (16), either at pH 6, or at pH 3, did not undergo oxidation with Ru^{VIII} species. However, the lower analog, N-benzyloxycarbonyl asparagine methyl ester, (15), on reaction with Ru^{VIII} at pH 6 resulted in a complex mixture.

The reaction of N-benzoyl arginine ethyl ester hydrochloride, either at pH 6, even for prolonged periods (100 h), as well as in phosphate buffer at pH 3 for 60 h, did not undergo oxidation with Ru^{VIII}. The presence of the guanidine residue in the protonated form precluded its oxidation. In a similar

manner, N^{α}-benzyloxycarbonyl lysine, (14), in phosphate buffer (pH~3) for 18 h, led to recovery of the starting material.

The work described above presents the outcome of endeavours pertaining to the reactions of the Ru^{VIII} species on each of the potentially susceptible coded α-amino acid side chains. This study was required for further developments relating to the application of Ru^{VIII} oxidations to peptides.

The results from the above study pertaining to the oxidation of coded amino acid side chains and summarized in TABLE S.I = TABLE C.I show that a reactivity pattern exists, namely, that whilst the most susceptible residue would be methionine, the others could perhaps be arranged in the following order of decreasing reactivity, under normal oxidation conditions : Trp, His, Tyr, Phe, Pro. In a similar manner, in pH 3 phosphate buffer, the order could be anticipated to be in a decreasing order : Trp, Tyr, Phe, His, Pro, Lys, Arg. These expectations have been realized on studies with di- and higher peptides.

Whilst the oxidation of BzPheOMe, BzPhe-PheOMe and BzPhe-Phe-PheOMe with Ru^{VIII} species under usual reaction conditions has been demonstrated already, leading to, respectively, BzAsp(β-OH)OMe, BzAsp(β-OH) - Asp-(β-OH)OMe and BzAsp(β-OH)-Asp(β-OH)-Asp(β-OH)OMe, in the present work the oxidation of N-^{t}butyloxycarbonyl phenylalanyl phenylalanine methyl ester, (21), under restrictive conditions, using 4 eq. of $NaIO_4$, resulted in the recovery of the starting material. However, the selective transformation of the tyrosine residue in N-benzoyl tyrosinyl phenylalanine methyl ester, (22), with Ru^{VIII}, even with a restricted time duration of 12h, gave only BzAsp(β-OH)-Asp(β-OH)OMe, (44, 74%) (CHART S.15 = CHART C.XXXVIII).

The reaction of N-benzoyl tryptophanyl leucine methyl ester, (23), with Ru^{VIII} under the usual conditions, gave, BzAsp(β-OH)-LeuOMe, (45, 58%). The expected preferential oxidation of the tryptophan residue was accomplished in the reaction of N-benzoyl tryptophanyl phenylalanine methyl ester, (24), with Ru^{VIII} using 18 eq. of periodate for 8 h leading to the formation of BzAsp-(β-OH)-PheOMe, (47, 66%) and the novel dipeptide N^{α}-benzoyl N^{ω}-formylkynurenyl phenylalanine methyl ester, (46, 13%). The BzAsp(β-OH)-PheOMe, (47), obtained in this manner is N-protected aspartame, a sweetening agent which is used widely (CHART S.16 = CHART C.XXXIX).

The tetrapeptide ZLeu-Pro-Leu-TrpOMe, (51), on treatment with 18 eq. of $NaIO_4$, is transformed with Ru^{VIII} during a shorter period of 8 h, cleanly, to ZLeu-Pro-Leu-AspOMe, (52), in 60% yields, without affecting the proline residue (CHART S.17 = CHART C.XL).

The susceptibility of the proline residue towards oxidation with Ru^{VIII} as a function of its placement in a peptide was illustrated using the dipeptide pair BzPro-PheOMe, (25), and BzPhe-ProOMe, (26). The reaction of (25) under the usual conditions, gave, BzPro-AspOMe, (49, 29%) and BzGlu-AspOMe, (48, 14%). In striking contrast, BzPhe-ProOMe, (26), under similar conditions, gave exclusively, BzAsp-ProOMe, (50, 62%). Thus, the N-benzoyl protecting group, when directly attached to the proline residue, as in the case of (25), makes it susceptible to oxidation. On the other hand, when the proline residue is in a peptide environment as in the case of (26), it is unaffected. This conclusion is supported from studies involving higher peptides (vide infra) (CHART S.18 = CHART C.XLI).

The oxidative transformations of dipeptides and a tetrapeptide

carried out thus far is summarized in TABLE S.2 = TABLE C.II.

Selectivity observed thus far and inferred from many of the experiments cited above, has enabled conclusions which are presented in TABLE S.3 = TABLE C.III.

Chromatographically homogeneous Cys-10 deleted signal hydrophobic segment of c-lysozyme, namely, the undecapeptide BocLeu-Val-Leu-Phe-Leu-Pro-Leu-Ala-Ala-Leu-GlyOBzl, (53), on treatment with Ru^{VIII} under normal conditions gave, cleanly, the expected specifically site altered undecapeptide, BocLeu-Val-Leu-Asp-Leu-Pro-Leu-Ala-Ala-Leu-GlyOBzl, (54), in quantitative yields. As anticipated, the proline residue in (53) was not oxidized. The (53)→(54) change demonstrates that the Ru^{VIII} methodology developed in the present work could be used for the specific alteration of higher peptides (CHART S.19 = CHART C.XLII).

The above conclusion was further tested by oxidation of melittin, a protein containing 26 amino acid residues and which is the principal toxic component of bee venom. The objective was to selectively oxidize the single tryptophan unit present in melittin to aspartic acid without affecting the other susceptible groups present, namely, lysine, threonine, proline, serine and arginine and, in addition, the unprotected amino end. Melittin, (55), was oxidized with Ru^{VIII} using 18 eq. periodate in pH 3 phosphate buffer for 8 h. Product analyses including optical data and amino acid analysis indicated the transformation of tryptophan to aspartic acid. The ORD pattern of the product was identical to that of the precursor, indicating retention of the secondary structure. The correlation of the amino acids on the basis of an analysis was satisfactory although not exactly as predicted (CHART S.20 = CHART C.XLIII).

In the present work, oxidative studies were carried out with selected unprotected α-amino acids in order to delineate preferences for the side chain oxidation compared to that of the free α-amino acid unit present and to reconcile conflicting reports in the literature pertaining to these oxidations. The oxidative studies were generally carried out at three pH values, namely, phosphate buffer (pH~3), aqueous media (pH ~6), and satd. $NaHCO_3$ (pH~ 9).

The reaction of tyrosine with Ru^{VIII} in presence of 18 eq. of periodate in MeCN - CCl_4 - phosphate buffer for 0.8 h at rt gave a 50% yield of aspartic acid. However, at pH 6 and pH 9, the exclusive product was malonic acid obtained, respectively, in 20% and 48% yields. In sharp contrast, phenylalanine, at pH 3 as well as at pH 6, gave only phenylacetic acid, respectively, in 76% and 43% yields. These results demonstrate the importance of the susceptibility of the aromatic moiety towards oxidation. The two sets of results cited above are rationalized on the basis of dissociation equilibria and the susceptibility of the aromatic moiety towards oxidation. At low pH, the α-amino acid moiety is largely protonated and the formation of aspartic acid from tyrosine can be explained on the basis of the selective oxidation of the p-hydroxyphenyl ring. However, in the case of phenylalanine, where the proclivity for such oxidation is highly diminished, the reaction course is governed by small amounts of free phenylalanine present, even at pH 3, which can be expected to undergo ready oxidation to phenylacetic acid. Thus, in the case of phenylalanine, the oxidation is at the free amino acid level, whereas, in the case of tyrosine, the oxidation involves not only the protonated α-amino acid residue leading to aspartic acid, but also, at higher pH, malonic acid, arising from preferential oxidation of the free amino acid unit followed by further oxidation of the p-hydroxyphenyl ring (CHART S.21 = CHART C.XLV).

The differences that exist between the pKa of the α-amino group (pKa 8.90) and the ω-amino function (pKa 10.28) of lysine has been taken advantage of to demonstrate preferential oxidation of the ω-amino moiety. Thus, lysine, either at pH 3, or at pH 6, gave, as the sole isolable product, glutaric acid monoamide in, respectively, 34% and 33% yields arising from the expected oxidation of the α-amino moiety to 2-keto 6-amino hexanoic acid, and then to 5-amino pentanoic acid, followed by cyclization, further oxidation and hydrolysis (CHART S.22 = CHART C.XLVI).

The results obtained from studies on the free amino acids tyrosine, phenylalanine, valine and lysine are presented in CHART S.23 = CHART C.XLVII.

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SECTION A : INTRODUCTION

The advent of cloning methodologies coupled with increasing capabilities in diffraction techniques have led to the development of intense interest currently in the understanding of the contribution of coded amino acid side chains, not only with respect to their specific functions, but also towards the generation of the unique structures that characterize enzymes. These enquiries, even within the limited span of a decade, have led to the elucidation of unexpected facets and molecular details pertaining to structure - activity correlations. These studies have shown that in the creation of the active surface of the enzyme designed to harbour the specific substrate, practically every one of the amino acid side chains has a role. Although it would be an awesome task to delegate to each and every one of the amino acid residues in a complex enzyme it's role in the creation of the unique functional system, it could, in principle, be accomplished. Such an understanding would have for reaching implications in practically every domain of molecular biology and would reinforce current notions pertaining to the selection and evolution of these systems.

Ironically, all these have become possible because of developments in the domains of organic chemistry. Currently, in the frontier areas of investigations related to enzymes, however, chemical methodologies thus far, if at all, have played very marginal roles. This is primarily as a result of endeavours to adapt successfully cloning methodologies to site specific side chain alteration on the one hand, and the lack of enthusiasm to use chemical tools arising out of uncertainities relating to effecting precise site changes chemically without affecting a large number of susceptible residues, on the other.

The thrust of the present endeavours is directed at the possible development of an oxidative methodology that could, as a result of extensive experimentation, prove versatile in bringing about specific side chain alterations amongst a number of susceptible residues, even in complex systems. Development of such methodologies would add a novel facet with respect to protein alterations and could even prove, in the long run, superior to cloning methodologies, both in terms of experimental arduors, and with respect to preparation of altered proteins in quantities.

The chemical literature abounds with pathways and pointers that might lead to accomplishment of such objectives. Over a number of years, experimental data has become available which deal with the chemistry of coded amino acids which, in several instances, involve the modification of the side chains.

A careful examination of the literature shows that this domain has not been reviewed. Consequently, it was considered most appropriate to glean from the literature information relating to transformations of coded amino acids brought about by oxidation. Such an account is presented in the following section.

SECTION B : BACKGROUND

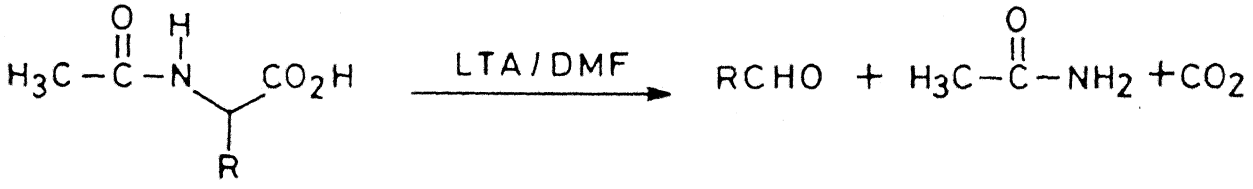
Of the 20 coded amino acids, 13 possess side chains that can be considered susceptible towards oxidizing agents. Logically, therefore, an oxidative methodology could provide maximum flexibility with respect to side chain alterations in peptides. Interestingly, the literature provides information pertaining to the oxidative transformation of as many as 11 of these side chains. As could be anticipated, the α-amino acid moiety is susceptible to oxidation in every case. In order to provide a comprehensive coverage, the oxidation of coded amino acids, irrespective of the site of oxidation, are summarized below and the amino acids are arranged in the alphabetical order.

ALANINE :

The decarboxylation of α-amino acids is one of the common reactions encountered in in vivo and in vitro systems. An appropriate illustration of this is the oxidation of a range of N-acetyl α-amino acids (alanine, glycine and valine) to the corresponding aldehydes brought about with LTA - DMF. This reaction, interestingly, leads to complex mixtures when oxidizable functions are present (phenylalanine and tryptophan) (CHART B.1)[1].

p-Toluenesulfenyl chloride brings about the transformation of α-amino acid esters to the corresponding α-keto compounds under mild conditions and in good yields. Thus, alanine, phenylalanine and cysteine are transformed to the corresponding α-keto esters which should show that this method is of general applicability. The transformation initially leads to the Schiff's base IV, which with Ph_3P supported on silica gel is transformed to the α-keto esters. Compound IV, in turn, arises via sulfur-substitution of a bis N-liganded precursor. Therefore,

CHART B-1



I II

R = H, $-CH_3$, $-CH(Me)_2$

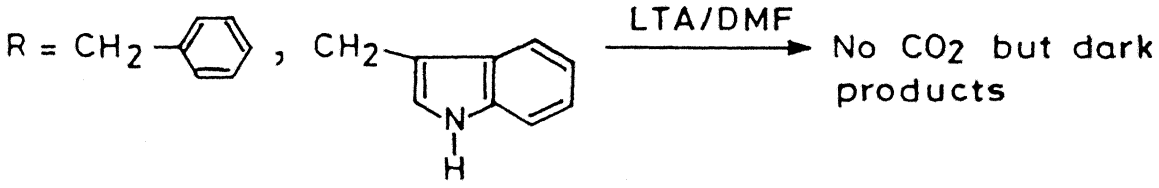
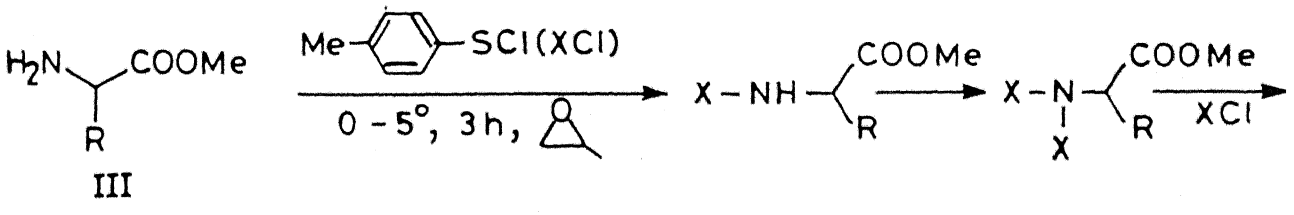
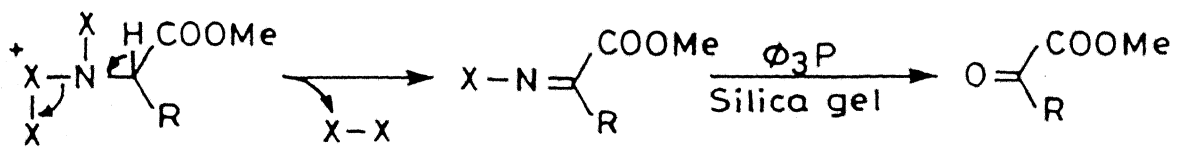


CHART B-2



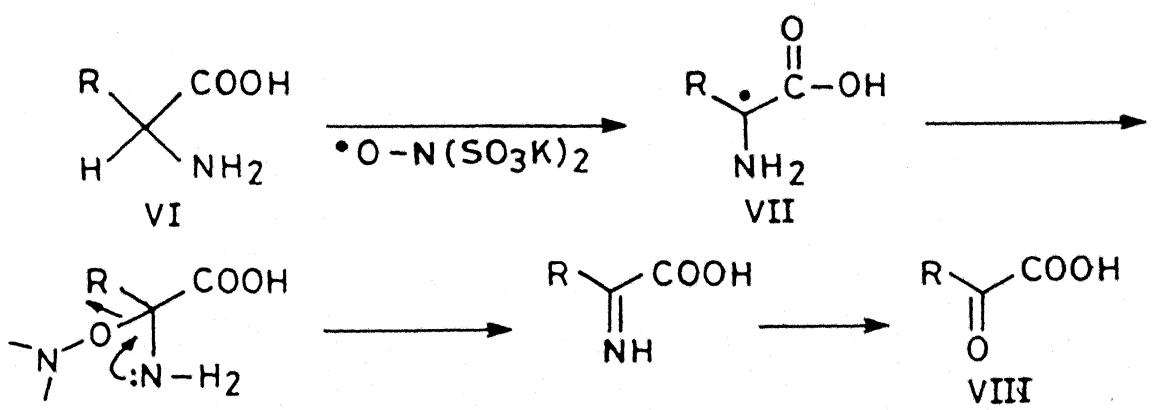
III



IV V

R = CH_3, CH_2Ph, CH_2SH

CHART B-3



VI VII VIII

R = H, CH_3, ^{i}Pr, ^{i}Bu, $(CH_2)_2COOH$, CH_2Ph

the formation of IV requires 3 mols of the reagent (CHART B.2)[2].

The α-amino acid unit presents 3 sites for hydrogen abstraction. Interestingly, Fremy's salt, $\dot{O}$-N$(SO_3K)_2$, specifically generates a radical centre at the tertiary carbon atom which, on combination with another molecule of Fremy's salt followed by fragmentation leads to, in sum, the oxidation of the α-amino centre to a Schiff's base. The latter suffers ready hydrolysis, leading to α-keto acids. The reaction takes place at pH ~ 10 and alanine, valine, glutamine and phenylalanine could be readily transformed to the corresponding α-keto acids (CHART B.3)[3].

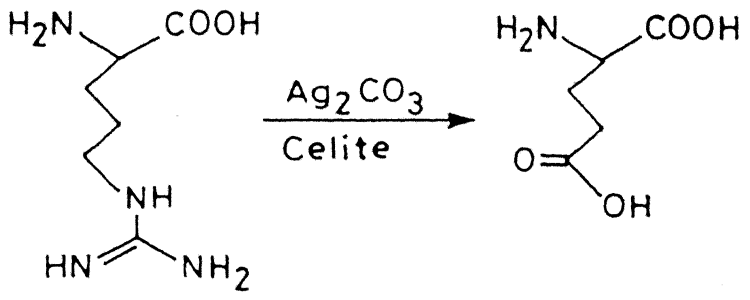
ARGININE :

Silver carbonate, supported on celite, brings about the oxidation of a variety of substrates, although not in a very specific manner. This aspect is exemplified by the oxidation of arginine which leads to a number of products, a noteworthy one amongst which is glutamic acid (CHART B.4)[4].

ASPARTIC ACID :

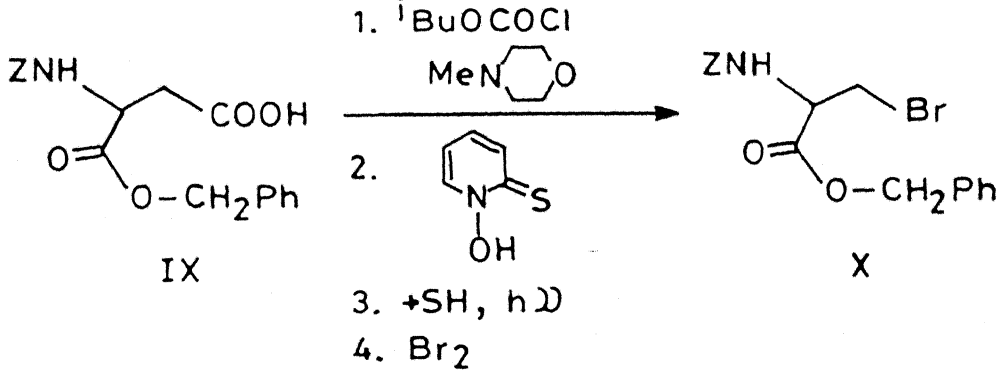
The oxidative decarboxylation of amino acids has been brilliantly taken advantage of in bringing about diverse transformations in the α-amino acid/peptide domain[5]. The transformation of the β-COOH of N,C-protected aspartic acid to the corresponding β-bromo compound (X) represents an elegant approach towards functionalization of amino acid side chains. Compounds of the type X offer possibilities for further elaboration. In a similar manner, the corresponding glutamic acid was also transformed to the γ-bromo compound. The mechanisms of these transformations are discussed later (CHART B.5).

CHART B-4



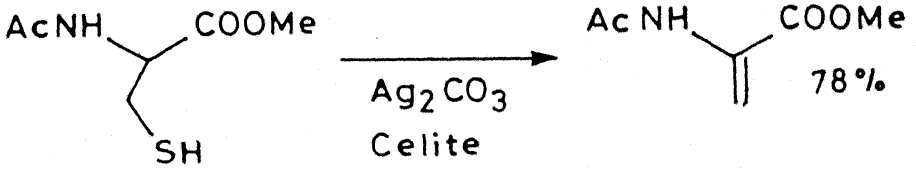
Arg Glu

CHART B-5



IX X

CHART B-6



XI XII

CYSTEINE :

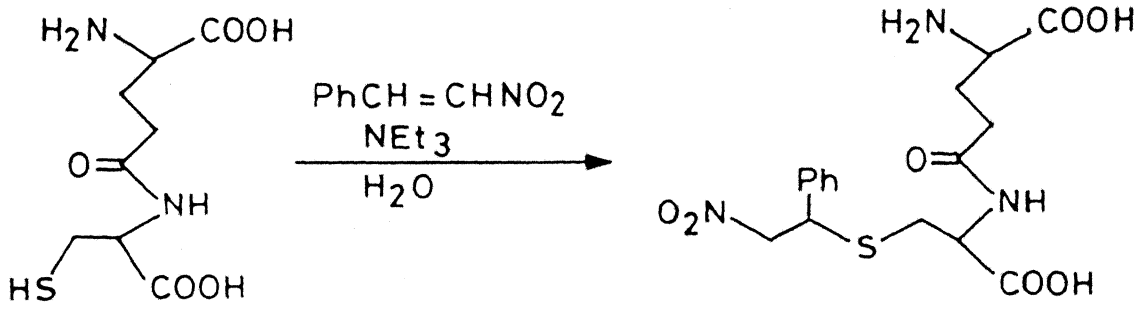
The preparation of N,C-protected dehydroalanine from the corresponding cysteine precursor, in 78% yields, represents perhaps one of the best routes to this class of compounds (CHART B.6)[6].

The acceptance of electrophilic substrates by the sulfhydryl group of cysteine can be construed as an oxidation. In this context, the acceptance of β -nitro styrene by the cysteine -SH, even in a peptide environment, is noteworthy. A practical application of this transformation would be the preparation of crystalline derivatives of proteins, thus making them amenable to X-ray analysis (CHART B.7)[7].

GLUTAMIC ACID :

The decarboxylation of side chain residues of amino acids leading to hydrocarbon residues represents a drastic change in the polypeptide profile. That such a change is possible is illustrated with the transformation of BocGln-GluOBzl to XIV. N,C-protected aspartic and glutamic acids can also be readily transformed to, respectively, alanine and α -amino butyric acid (CHART B.8)[5].

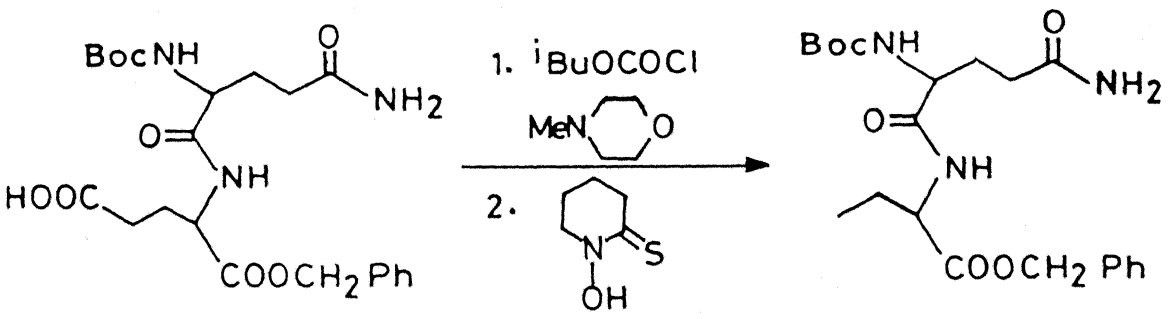
The use of aspartic acid and glutamic acid in peptide synthesis calls for the protection of the side chain carboxyl unit. This would necessitate the removal of such groups upon completion of the peptide synthesis. This could be readily accomplished in the event the terminal residues are phenyl hydrazides such as in XV and XVI via brief oxidation in aq. acetic acid with MnO_2 (CHART B.9)[8].



L - Glutathione

XIII

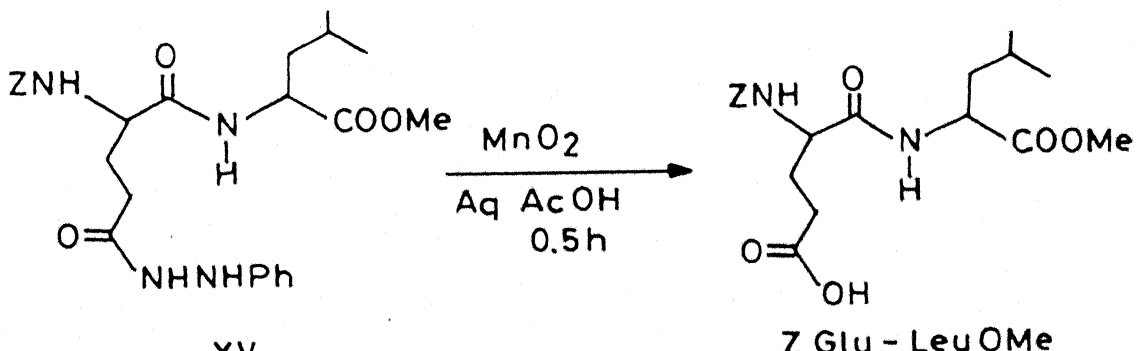
CHART B-8



BocGln - GluOBzl

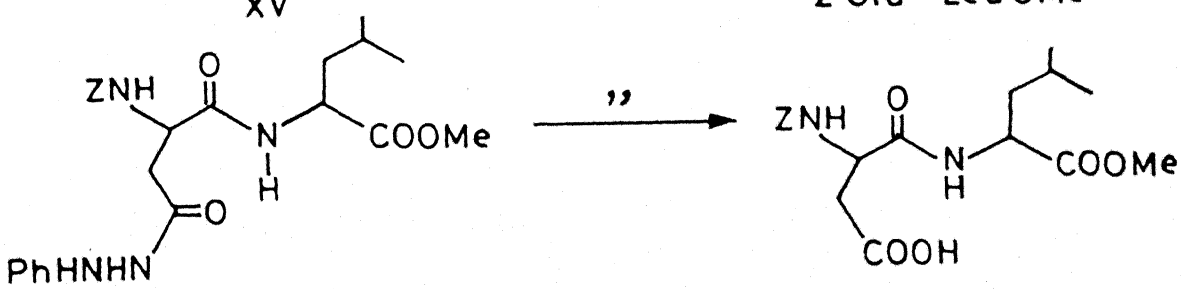
XIV

CHART B-9



XV

Z Glu - LeuOMe



XVI

ZAsp - LeuOMe

HISTIDINE :

Perhaps more than any coded amino acid, histidine plays very crucial roles in the functional system. This notion is supported by the fact that this residue is present at the active site of nearly every enzyme. The premier pathway in histidine catabolism is via oxidation. Consequently, the chemical and photochemical oxidation of histidine has attracted much attention. In view of its high susceptibility, the imidazole moiety of histidine is generally the primary target of oxidizing agents. However, procedures are available to effect preferential oxidation of the α -amino acid unit.

A variety of reagents transform protected as well as free histidine to aspartic acid or its derivatives. These reagents are hydrogen peroxide, singlet oxygen and ozone. In some of these transformations the corresponding asparagines are implicated and indeed have even been isolated in the sensitized oxidation of N,C-protected histidine. The reaction of histidine with periodate is particularly noteworthy in the sense that here the imidazole unit is retained at the expense of the α -amino acid grouping (CHART B.10)[9].

In liver and bacteria, histidine is rapidly metabolized to glutamic acid via a series of unusual changes involving the imidazole 4-hydroxylation as a key step. The latter event, followed by subsequent changes takes place upon reaction of the N,C-protected histidine XXI with 4-^{t}butyl iodoxybenzene leading to the γ -formamido glutamine XXV (CHART B.11)[10].

LYSINE :

The transformation of peptide side chains calls for methodologies that avoid harsh reagents and reaction conditions. In this context, electrolyti-

CHART B-10

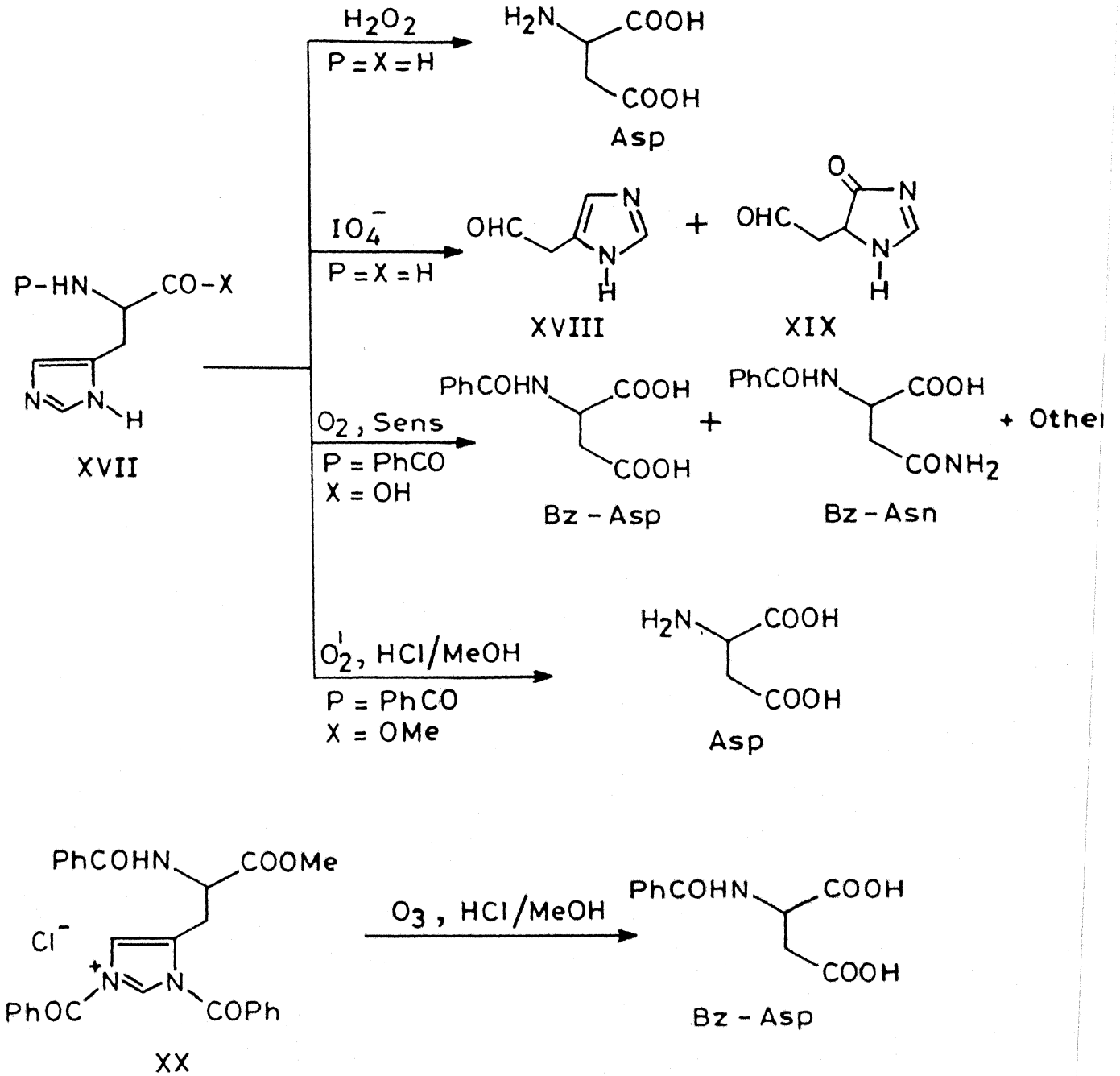

H2O2
P = X = H
H2N
COOH
COOH
Asp
IO4−
P = X = H
OHC
N
N
H
+
OHC
O
N
N
H
XVIII
XIX
P-HN
CO-X
N
N
H
XVII
O2 , Sens
P = PhCO
X = OH
PhCOHN
COOH
COOH
Bz - Asp
+
PhCOHN
COOH
CONH2
Bz - Asn
+ Other
O2, HCl/MeOH
P = PhCO
X = OMe
H2N
COOH
COOH
Asp
PhCOHN
COOMe
Cl−
N
N
PhOC
COPh
XX
O3 , HCl/MeOH
PhCOHN
COOH
COOH
Bz - Asp

CHART B-11

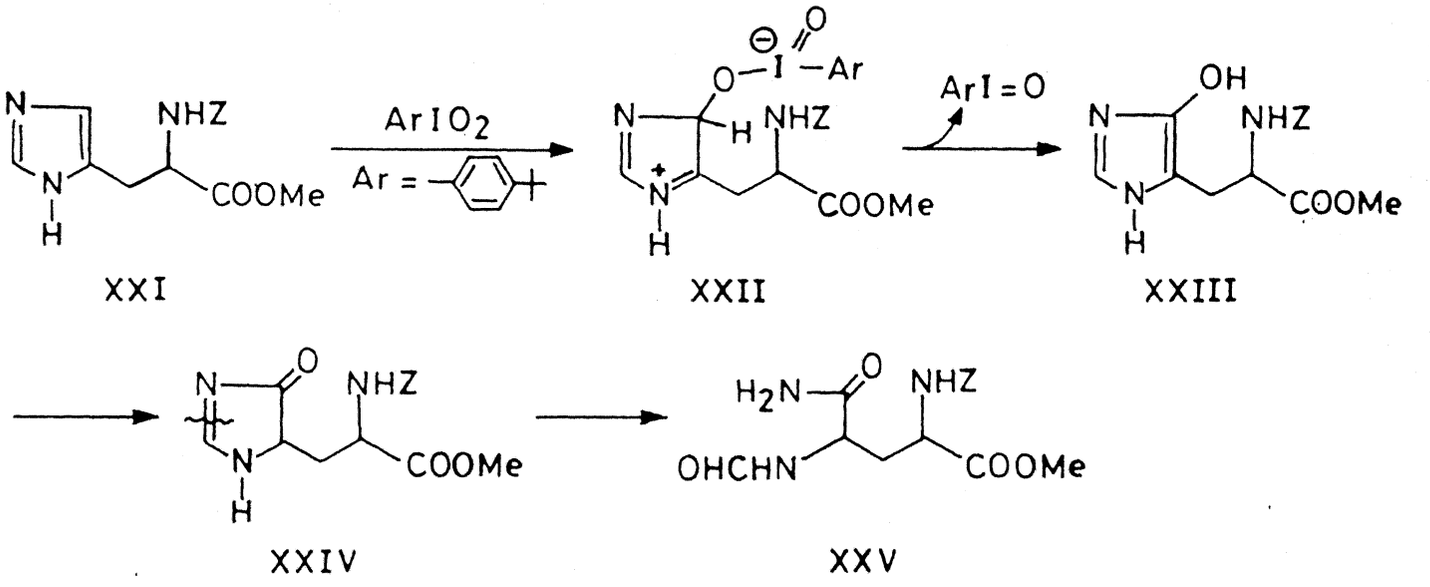
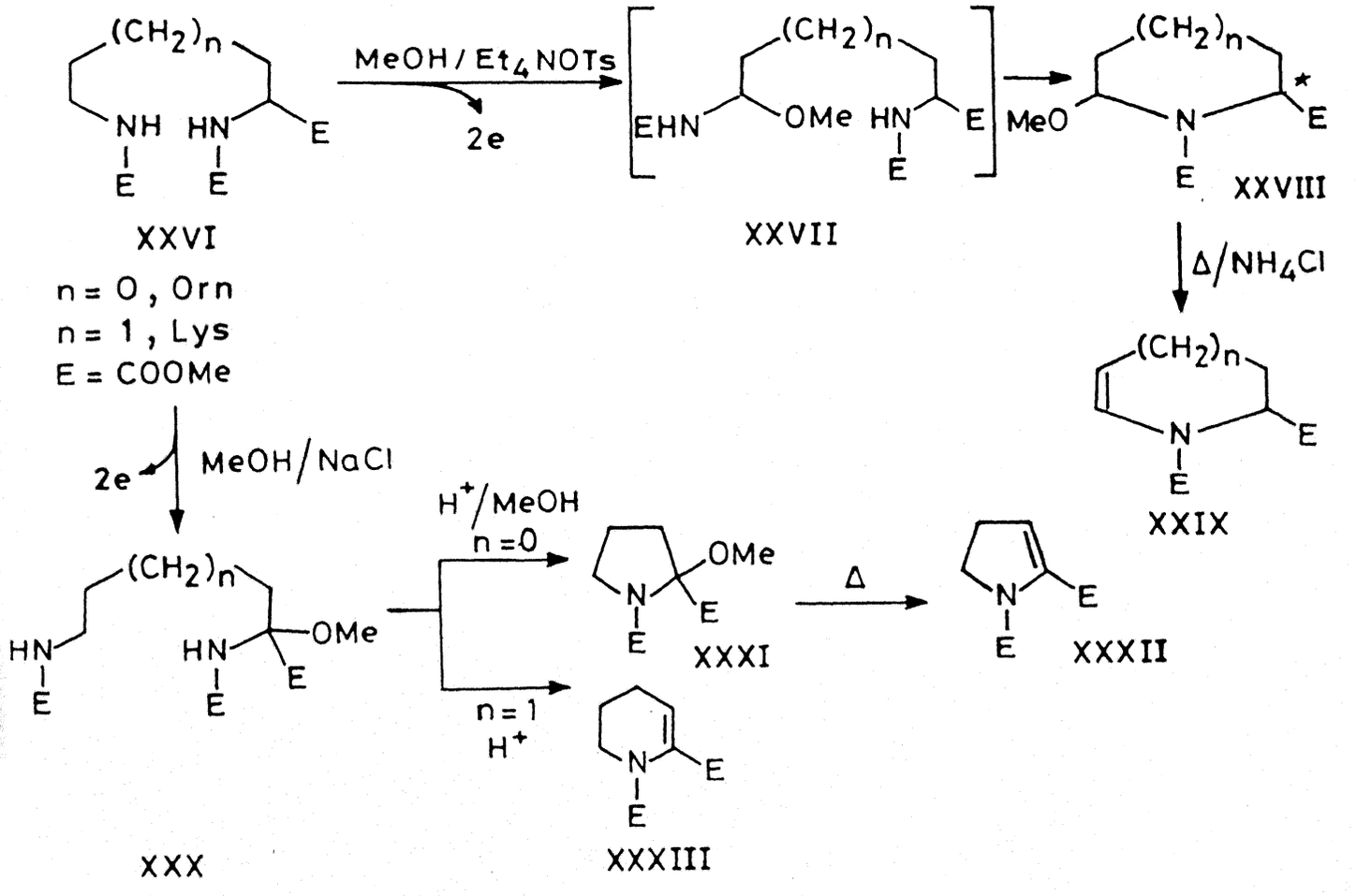


CHART B-12



cally mediated redox processes have found favour in recent times[11]. The versatility of these methods is illustrated with the electrolytic oxidation of lysine and ornithine. The ester urethanes of lysine and ornithine, XXVI, on electrolytic oxidation in MeOH-Et_4NOTs lead to specific ω-methoxylation and the resulting intermediate XXVII undergoes cyclization and loss of MeOH giving rise to, respectively, chiral δ^2-pyrolines and tetrahydropiperidines (XXIX). Alternatively, electrolysis in MeOH-NaCl leads to selective α-methoxylation, eventually giving rise to achiral 2-carbomethoxy δ^2-compounds XXXII and XXXIII. In a peptide environment, it is likely that intermediates represented by XXVII would lead to carboxaldehydes (CHART B.12)[12].

METHIONINE :

Methionine and cysteine are the most susceptible substrates towards oxidizing agents and electrophiles. Both these reactions contribute substantially to the chemical transformations of these residues. There are several interesting points associated with the oxidation of methionine. The primary oxidation leads to a chiral sulfoxide and considerable asymmetric induction can be incorporated in this change. The sulfoxide, in turn, offers possibilities for further oxidation. The sulfones thus obtained possess proximate active hydrogens and further oxidation is possible that would result either in degradation, or the loss of the methyl ligand.

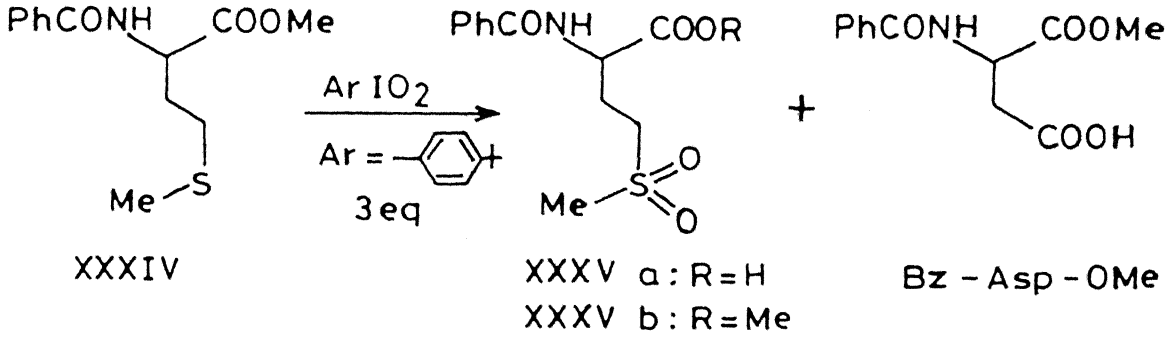
A careful study of the reaction of N,C-protected methionine with the ozone equivalent, 4-^{t}butyl iodoxybenzene has enabled the illustration of the occurrence of all these possibilities. Thus, the reaction of N,C-protected methionine, XXXIV, with 4-^{t}butyl iodoxybenzene gives rise to the sulfone XXXV b and the sulfoxide XXXVI b via unexceptional pathways. The formation of

BzAsp (β-OH)OMe and homocysteic acid XXXVII in this reaction can be attributed to carbon oxidations of XXXV b referred to earlier. In the formation of these products, there is a similarity with the oxidation of dibenzylsulfide with ozone[13].

The formation of the sulfone acid XXXV and the sulfoxide acid XXXVI a was unexpected and their formation is rationalized on the basis of intramolecular cyclization of the initially formed sulfoxide XXXVI b followed by hydrolysis and further oxidation. The oxidation with 4-^{t}butyl iodoxybenzene of the methionine side chain is general and is exemplified by the XXXVIII → XXXIX change and similar transformations with ZGly-MetOMe (CHART B.13)[14]. The range of products arising from oxidation of methionine XXXIV with 4-^{t}butyl iodoxybenzene is rationalized in CHART B.14. The initially formed sulfoxide XXXVI b could undergo cyclization to provide a reactive intermediate which, on opening and further oxidation, could lead to XXXV b. Compound XXXV b is the expected product of oxidation of compound XXXVI b. In many ways iodoxy benzene and 4-^{t}butyl iodoxybenzene mimic the properties of ozone. The further transformations of XXXV b involving the two proximate C-H bonds can be understood in terms of insertion of the reagent to these groupings followed by further fragmentation (CHART B.14)[14]. The intramolecular cyclization of the initially formed sulfoxide generating a reactive intermediate is also encountered on oxidation of ZCys (S-Bzl)OMe with 4-^{t}butyl iodoxybenzene giving rise to the expected acids and esters of the corresponding sulfoxide and sulfone.

N-protected methionines can be transformed to the corresponding sulfoxides using H_2O_2/$NaIO_4$/$NaBO_3$/NCS/Chloramine T. A detailed study of the selectivity of these reagents on a single amino acid substrate has shown that $NaIO_4$ and $NaBO_3$ yield preferentially the sulfoxide wherein complications

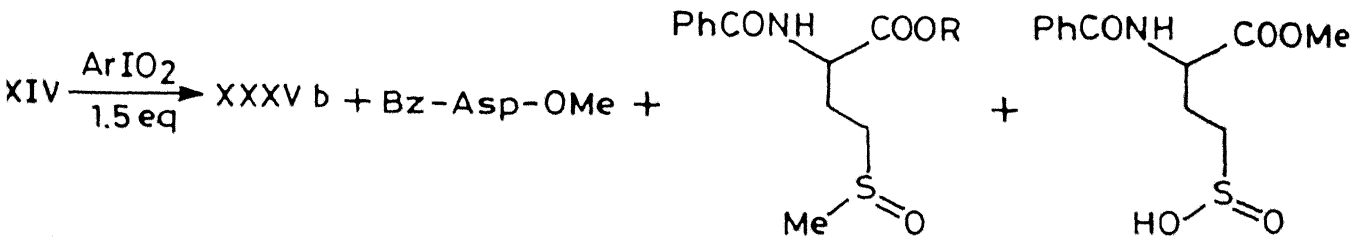
CHART B-13



XXXIV

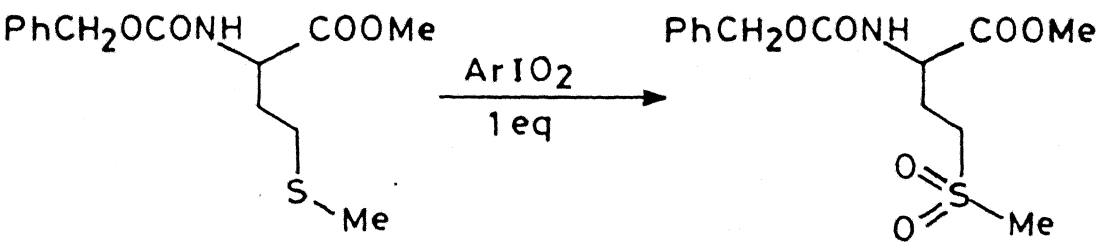
XXXV a : R = H
XXXV b : R = Me

Bz - Asp - OMe



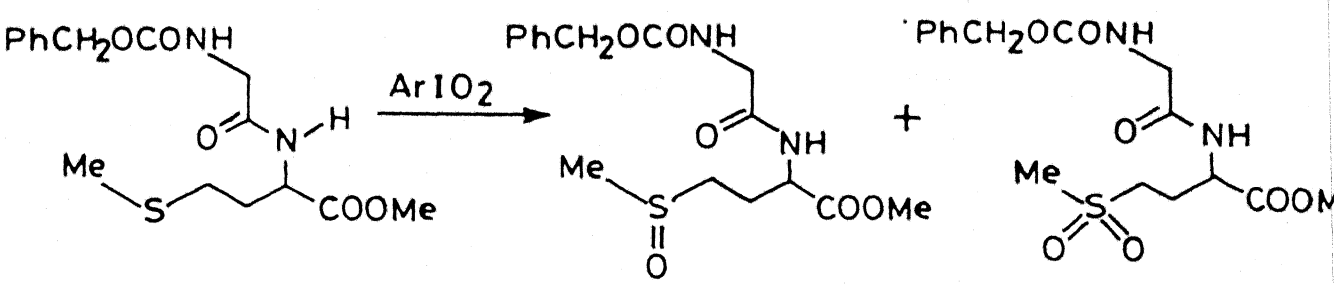
XXXVI a : R = H
XXXVI b : R = Me

XXXVII



XXXVIII

XXXIX

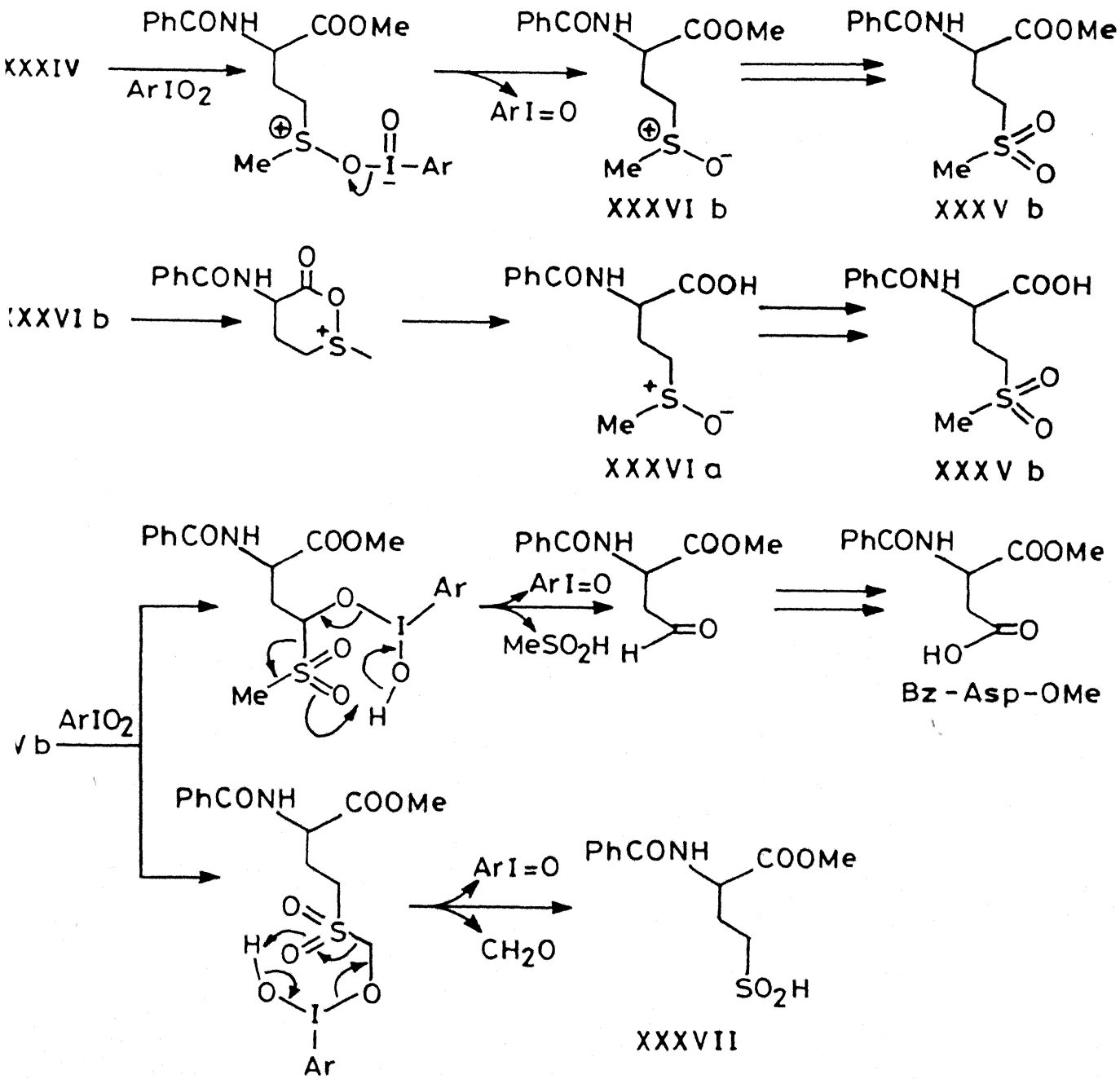


Z Gly - MetOMe

XL

XLI

CHART B-14


XXXIV
ArIO2
PhCONH
COOMe
Me
S
O
I
Ar
ArI=O
XXXVI b
XXXV b
XXXVI a
XXXV b
COOH
Bz-Asp-OMe
MeSO2H
HO
CH2O
SO2H
XXXVII

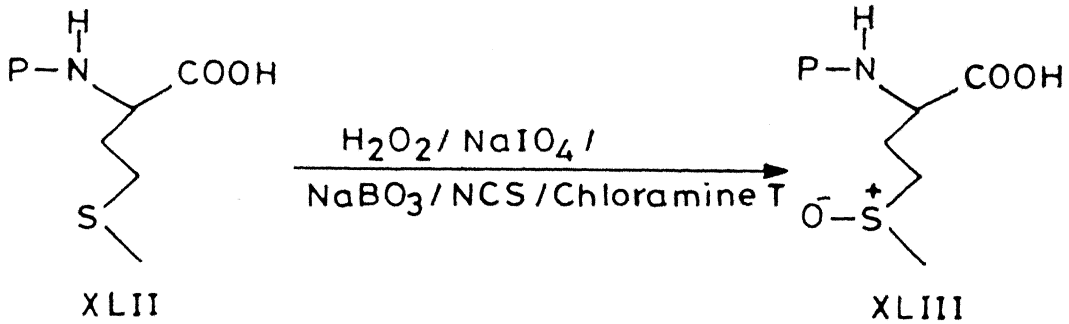
arising out of further oxidations are minimum. The methionine sulfoxide XLIII formed was used in the synthesis of the peptide kassinin (CHART B.15)[15].

A careful study of the reaction of methionine with iodine has brought to light novel chemistry, a particularly noteworthy feature of which is the formation of the rather uncommon N-S bond. The iodonium salt which is initially formed can undergo hydrolysis to the sulfoxide XLVI, or could lead to the extremely interesting cyclic system XLIV arising from nucleophilic displacement of a N-iodinated intermediate. Compound XLIV, in turn, then loses iodine to initiate another set of such reactions giving rise to the zwitterionic product XLV (CHART B.16)[16].

A measure of protection from oxidation can be provided to methionine residues present in peptides in an exceptionally easy manner via oxygen transfer from dimethyl sulfoxide in aqueous HCl at rt. The resulting sulfoxides are not susceptible to attack by electrophilic reagents, thus enabling the peptide modification at other sites such as in tryptophan and tyrosine. The methionine S-oxide side chains could be transformed to the original methionine residues, again by oxygen transfer using dimethyl sulfide in aqueous HCl at a pH range of 4-10. The forward and reverse oxygen transfer has been demonstrated in bovine α-lactalbumin, ribonuclease, insulin and ACTH. The procedure does not affect any of the other coded amino acids including tryptophan and free cysteine (CHART B.17)[17].

Another method that is effective in the protection of the methionine side chain towards oxidizing agents and electrophiles involves alkylation with the ^{t}butyl cation. Thus, Met-Tyr can be transformed to the S-^{t}butyl compound XLIX which is resistant to other transformations at the α-site. The practical

CHART B-15



P = +OCO, $PhCH_2OCO$,
p-OMePh CH_2OCO

CHART B-16

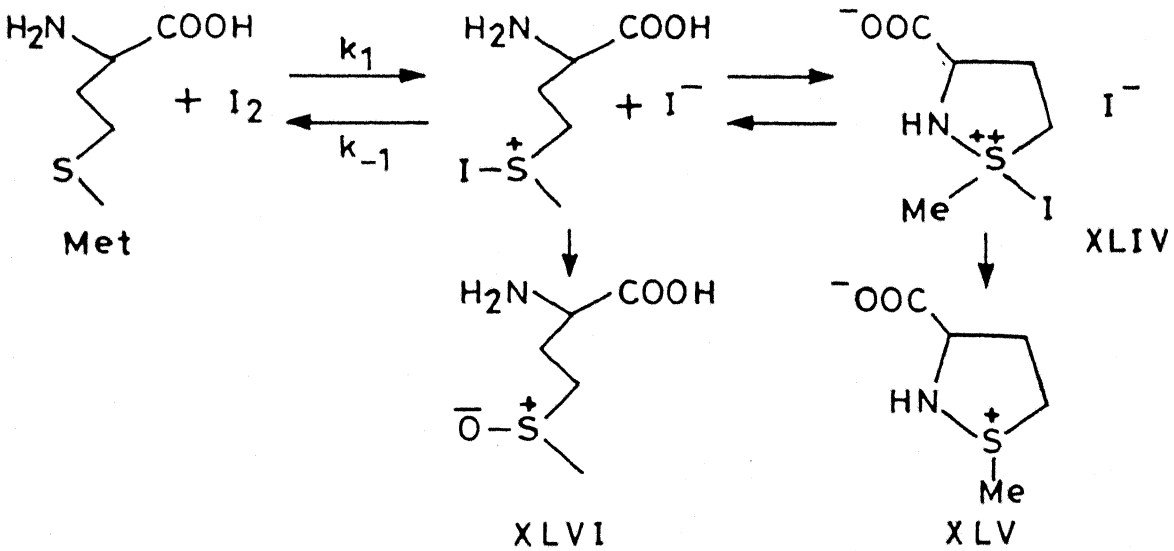
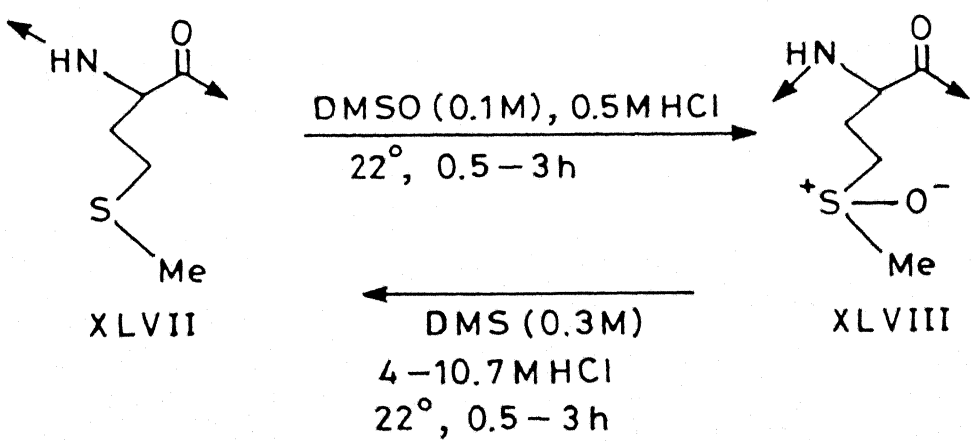


CHART B-17



utility of this protection is illustrated by the fact that XLIX can specifically iodinate the tyrosine residue leading to L which, in turn, can be converted to the free methionine system LI by reaction with a range of nucleophiles. Considering the fact that the sulfurs in both cysteine or methionine are the most susceptible sites for reaction with oxidizing agents or electrophiles, their protection is of importance in peptide modification (CHART B.18)[18].

The use of oxidative decarboxylation in peptide transformation has been illustrated earlier with focus on side chain carboxyl residues. Such changes could also be readily brought about at the carboxyl end of a peptide as illustrated with BocLeu-MetOH, the reaction of which with ^{i}BuOCl in presence of N-methyl morpholine, reaction with N-hydroxy pyridine 2-thione-triethyl amine and photolysis in the presence of ^{t}butyl mercaptan leads to the interesting compound LIV. The overall transformation involves the reaction of the initially formed mixed anhydride with N-hydroxy pyridine 2-thione leading to LII which, in turn, generates the carboxyl radical mediated by photolytically produced ^{t}Bu-S$^{\cdot}$. The radical intermediate LIII then could suffer loss of CO_2 and abstraction of hydrogen from ^{t}butyl mercaptan to initiate a second chain of reactions leading to the observed product. This highly efficient methodology has been demonstrated to have wide applicability. Thus, substrates such as phenylalanine, aspartic acid, methionine, serine, threonine, proline and arginine undergo decarboxylation to give products of a different reaction profile. This procedure would find applicability in the modification of C-terminal residues (CHART B.19)[19].

PHENYLALANINE :

The transformation of N-protected α-amino acids to the corresponding aldehydes was illustrated in CHART B.1 using LTA as the oxidizing agent. This

reaction can be even more readily accomplished with the unprotected α-amino acid unit using a variety of reagents such as hypochlorite, hydrogen peroxide, Chloramine T, ninhydrin and alloxan (CHART B.20)[20].

The specific p-hydroxylation of the phenylalanine side chain to tyrosine represents a very important biological transformation. This reaction has been extensively investigated leading to the development of highly sophisticated in vitro mimics one of which is presented in CHART B.21. The process of p-hydroxylation here is initiated by the tetrahydropterin, LVII, which, on reaction with Fe^{III} leads to a duplex which, in turn, by an intramolecular electron transfer generates a Fe^{II} complex capable of accepting triplet O_2. The oxygen complex thus formed is transformed to LIX involving the reoxidation of Fe^{II} to Fe^{III} with concomittant reduction of the oxygen. This transformation is also associated with the reduction of the radical cation centre in LVIII at the expense of the 2-amino pyrimidine moiety which, in turn, is transformed to a quininoid system, LIX. Complex LIX is then transformed to the symmetrical duplex LX involving the oxidation of the ligand pterin and the reduction of the oxygen radical anion to hydrogen peroxide. The hydrogen peroxide thus formed is liberated from the complex to give LXI and the hydrogen peroxide is available for specific p-hydroxylation of phenylalanine to tyrosine, the radical substitution producing equivalents of two hydrogens which is able to quench the diradical LXI leading to LXII. Compound LXII, in turn, undergoes intramolecular electron transfer and the Fe^{II} centre thus created is able to pick up another unit of oxygen to start the cycle all over again (CHART B.21)[21].

Interestingly, OH radicals in presence of Fe^{II} can bring about the Phe → Tyr change. Thus, the Phe → Tyr transformation is possible with H_2O_2, either in presence of $FeSO_4$ or Fe-porphyrin (CHART B.22)[22].

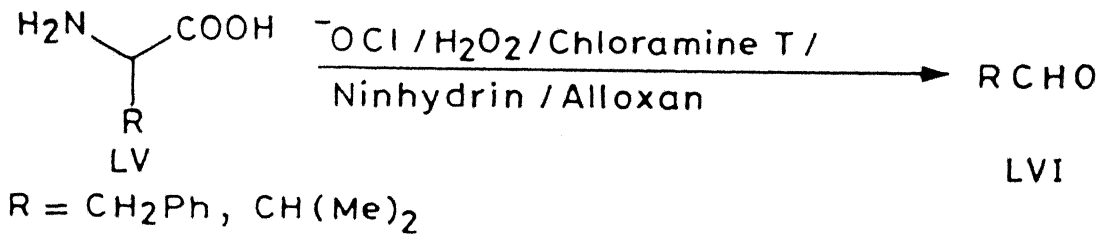
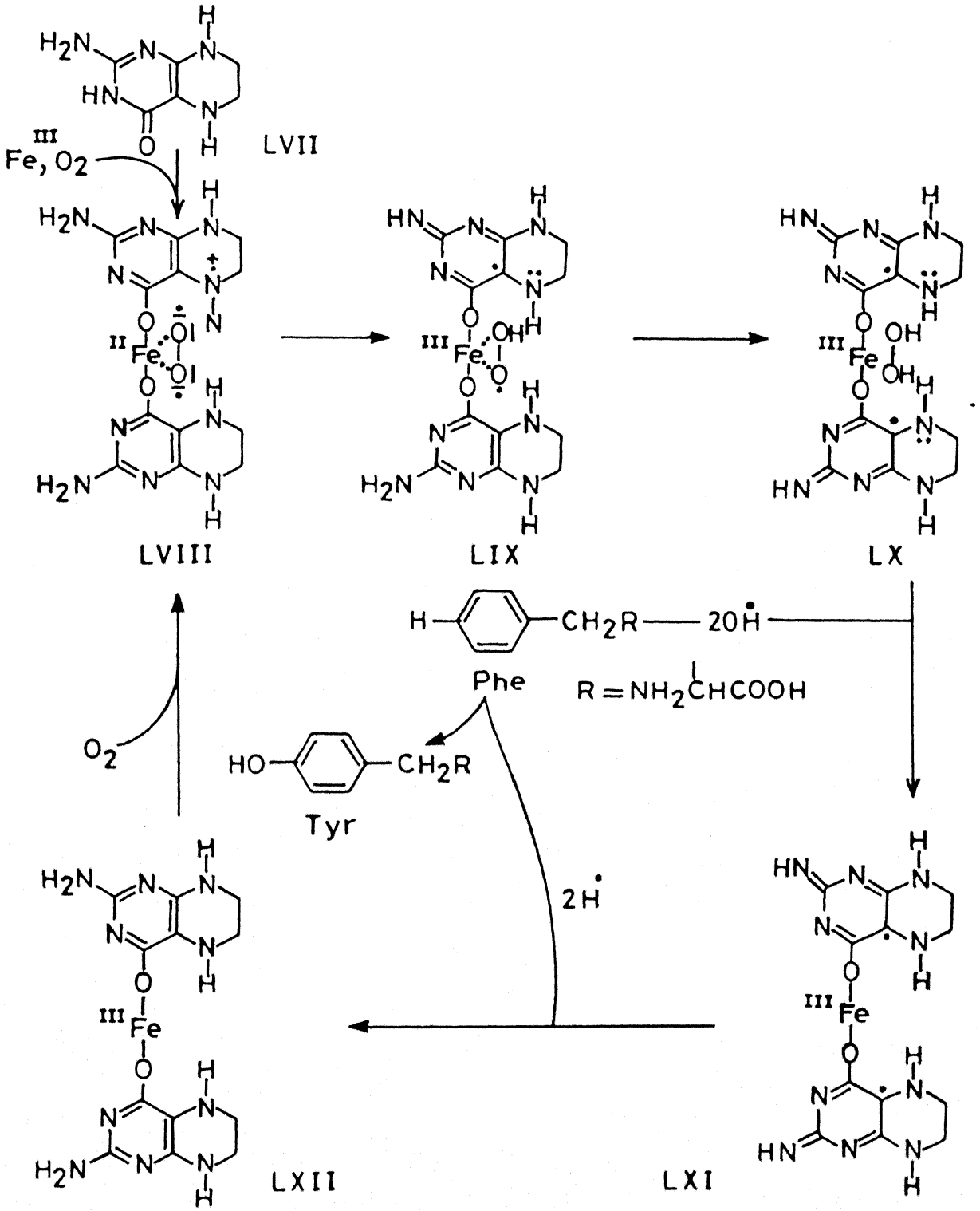

H_2N
COOH
R
LV
$^-OCl / H_2O_2$ / Chloramine T /
Ninhydrin / Alloxan
RCHO
LVI
$R = CH_2Ph$, $CH(Me)_2$

CHART B-21


LVII
Fe^{III}, O_2
LVIII
LIX
LX
$H-C_6H_4-CH_2R$ — $2\dot{O}H$
Phe
$R = NH_2CHCOOH$
O_2
HO — C_6H_4 — CH_2R
Tyr
$2\dot{H}$
LXII
LXI

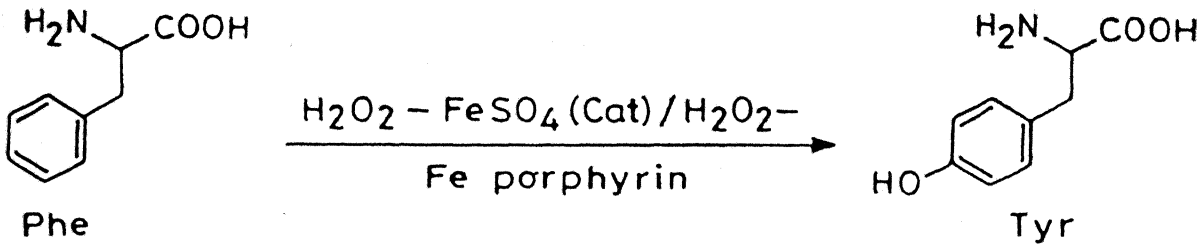


CHART B-23

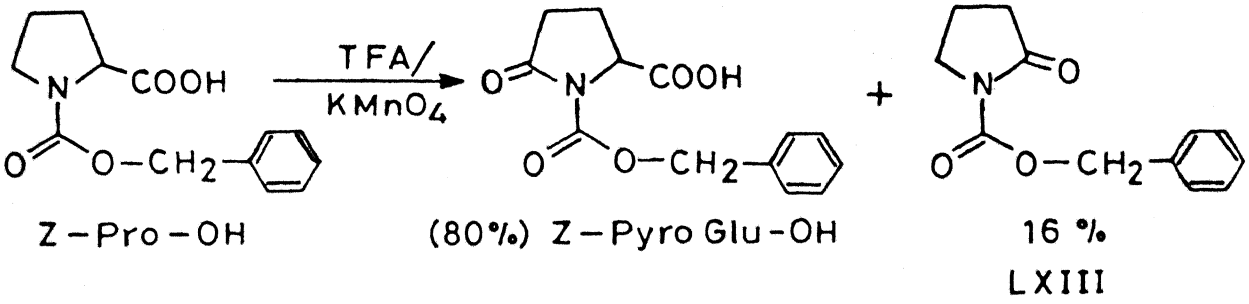
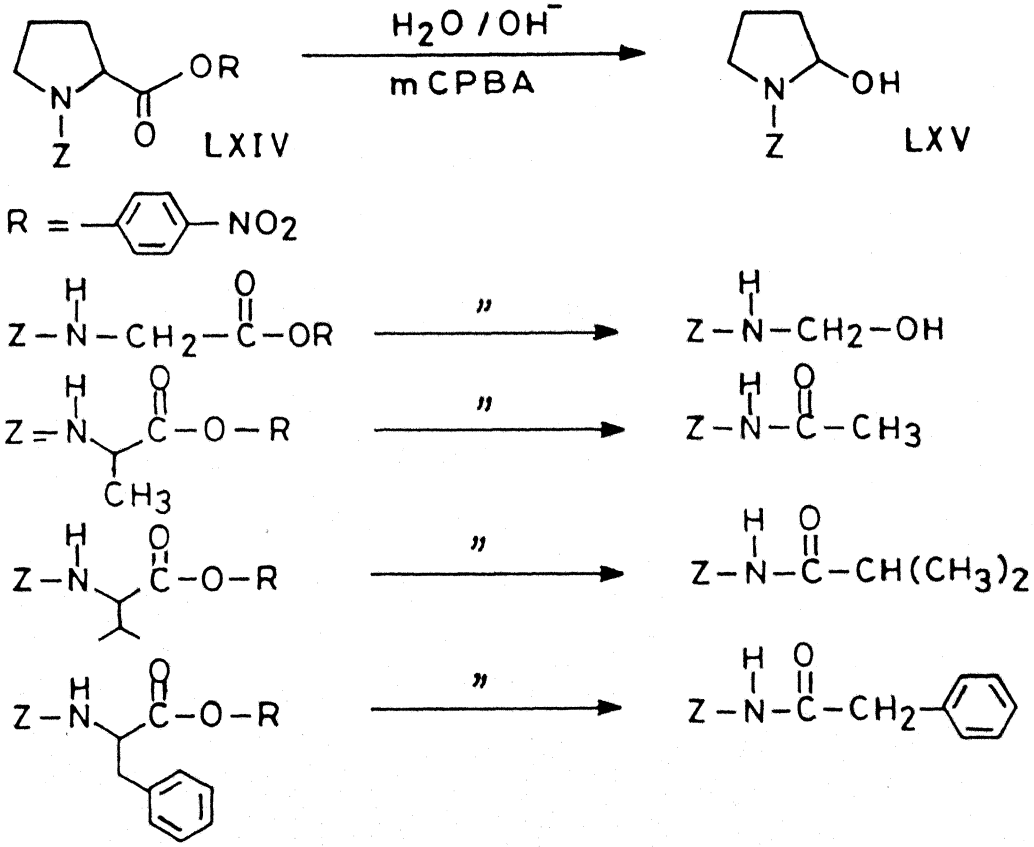


CHART B-24



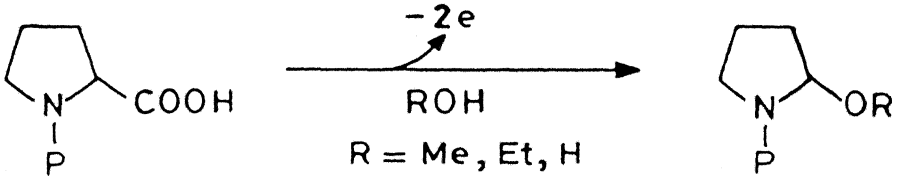
PROLINE :

N-Acylated prolines in their reactivity profile are markedly different from other similarly protected amino acids in the sense that as a consequence of the reduced exo π -bond order, the proximate C-H bonds are more reactive. This finds illustration in the transformation of ZPro-OH to the corresponding pyroglutamic acid in 80% yields with the oxidative decarboxylation leading to 2-pyrolidone, LXIII, as the minor product. The importance of N-acylation is brought out by the fact that proline itself is not susceptible to such oxidations (CHART B.23)[23].

An elegant methodology for bringing about the CHCOOH → CHOH/CO transformation of N-protected amino acids is via mCPBA oxidation of the corresponding p-nitrophenyl esters. Interestingly, in the case of proline and glycine, the transformation leads to the corresponding hydroxyl compounds via Baeyer-Villiger oxidation. However, in the case of alanine, valine and phenylalanine, the products are amides arising from, the lone pair mediated fragmentation of the intermediate peresters followed by hydrolysis of the resulting Schiff bases (CHART B.24)[24].

The electrolytic oxidative decarboxylation of proline in alcohols provides an useful method for the preparation of 2-alkoxypyrrolidines (CHART B.25)[25].

CycloPro-Pro and CycloPro-Gly - readily derived diketopiperazines - undergo sensitized oxygenation leading to hydroperoxides LXVII and LXVIII. Interestingly, N-AcPro-GlyOEt, which lacks the bicyclic system, does not undergo photo-oxygenation (CHART B.26)[26].



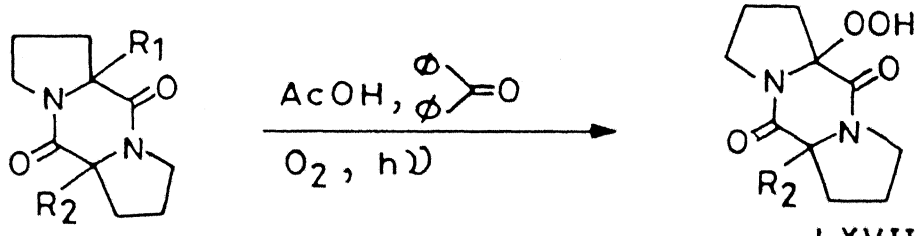
P-Pro

P = $PhCH_2-OCO$, PhCO

$COCH_2NHCH_2CO_2Bzl$, H

LXVI

CHART B-26



L, L-cyclo Pro-Pro

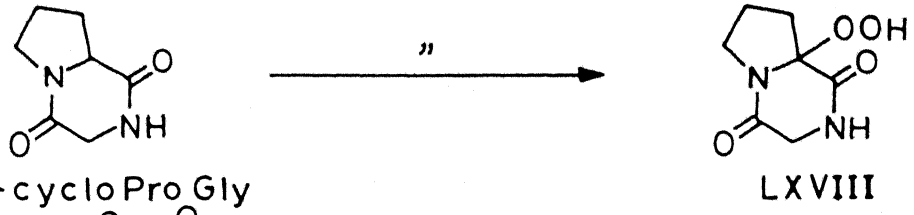
R_1 = H

R_2 = H

LXVII

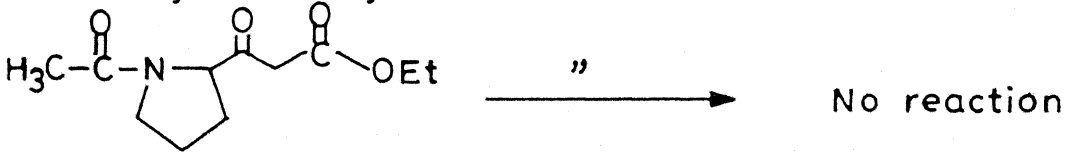
R_2 = H, 51%

R_2 = OOH, 46%



L-cyclo Pro Gly

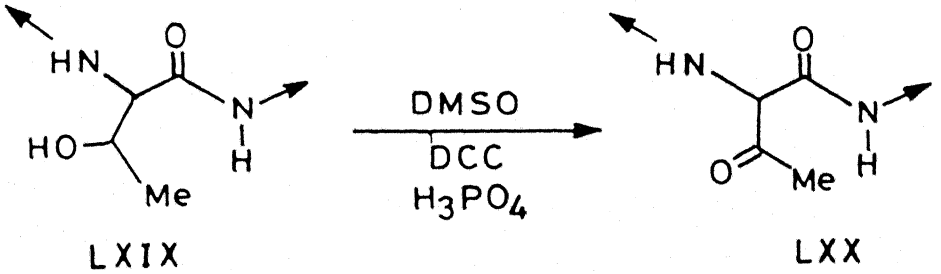
LXVIII



No reaction

N-Ac-Pro Gly OEt

CHART B-27



LXIX LXX

THREONINE :

Of the number of methods available for threonine oxidation, the DMSO - DCC procedure has been demonstrated to be applicable in the peptide area. Interestingly, cleavage at the carboxyl end can be effected via phenyl hydrazones in the pH range of 3-4 (CHART B.27)[27].

TRYPTOPHAN :

The amino acid tryptophan is so elegantly crafted that the spectrum of activity exhibited by this amino acid encompasses a very wide range of processes involved in life systems. Thus, tryptophan is involved in the tertiary structures of enzymes, in the biosynthesis of practically every one of the indole alkaloids, in the formation of neurotransmitters and in the production of highly coloured materials that constitute natural pigments. One of the earliest attempts at peptide modification was targeted at tryptophan residues, the chief attraction of such an effort being that this amino acid is present in enzymes in smallest numbers.

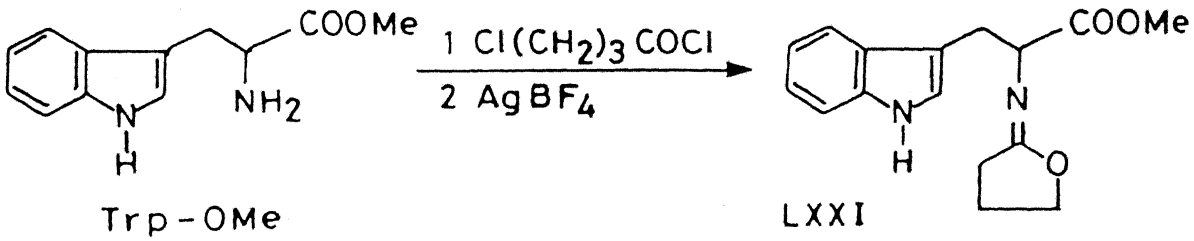
The chemistry of tryptophan is associated with the α-amino acid moiety that is common to all coded amino acids and the indole ring which has immense versatility to interact with practically every type of reactive intermediate. In addition, the α-amino group is so positioned in tryptophan that tricyclic intermediates arising from intramolecular cyclization onto the indole residue become common and possible.

The reaction of tryptophan methyl ester with 4-chlorobutyroyl chloride followed by treatment with silver fluoborate provides the interesting, completely N^{α}-protected LXXI. In the latter compound, the nucleophilic activity is reposed

on the α-amino nitrogen and, consequently, the reaction of this compound with alkylating agents leads to specific N-alkylation and the protecting group can simultaneously be removed on treatment with $KHCO_3$. This method, therefore, enables the specific alkylation of the side chain nitrogen (CHART B.28)[28].

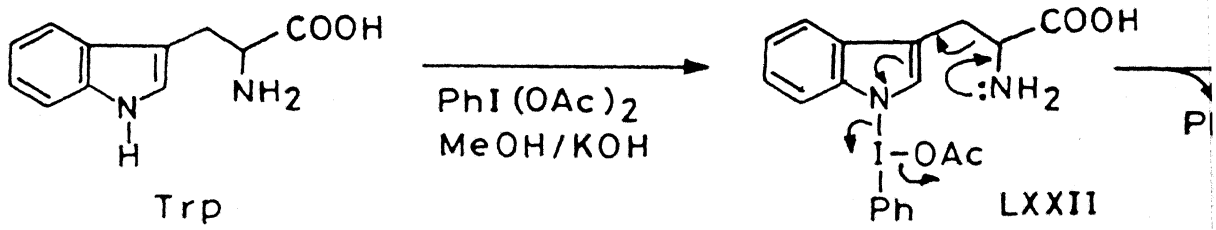
The nitrogen activity profile gets inversed in presence of alkali since it can generate the conjugate base of the indole nitrogen in sufficient concentrations to produce activity. This is best illustrated by the treatment of tryptophan with phenyl iododiacetate in presence of methanolic KOH leading to the total deletion of the indole side chain which can be isolated as the 3-methoxymethyl derivative LXXIV. In this unusual reaction, the electrophilic intermediate LXXII arising from the acceptance of the reagent at the indole nitrogen suffers fragmentation initiated by the α-NH_2 unit. N-terminal tryptophans can be degraded by this procedure. For example, Trp-Ala, Trp-Phe and Trp-Leu undergo ready fragmentation resulting in the formation of LXXIV. The fate of the peptide residue has not been clearly established. It is thought that the imino compound arising from fragmentation undergoes hydrolysis, oxidation and decarboxylation to provide the adjacent residue represented by LXXVI. The reaction needs a basic α-amino acid residue since N-protected tryptophans do not interact with the reagent. It is possible, however, that such N-protections that do not deplete the basicity of the α-amino group such as trityl or benzyl would undergo fragmentation (CHART B.29)[29].

The benzylic methylene unit present in tryptophan and attached to the indole 3-position can be specifically oxidized although most oxidizing agents attack the indole ring. An exception is DDQ which transforms N-protected tryptophan or the corresponding methyl ester LXXVII to LXXIX which suffers very ready cyclization giving rise to the natural product pimprinine, LXXVIII.

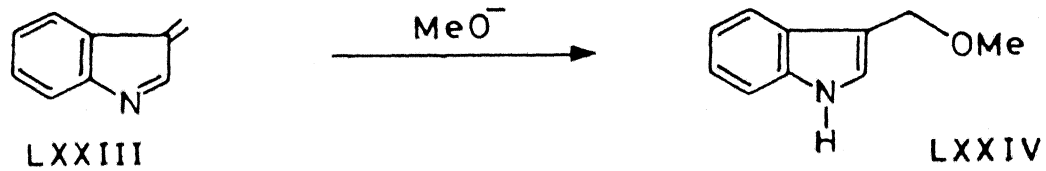


Trp-OMe LXXI

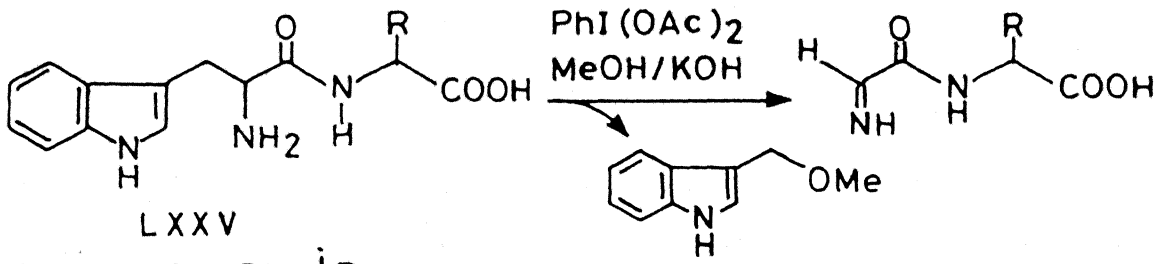
CHART B-29



Trp LXXII

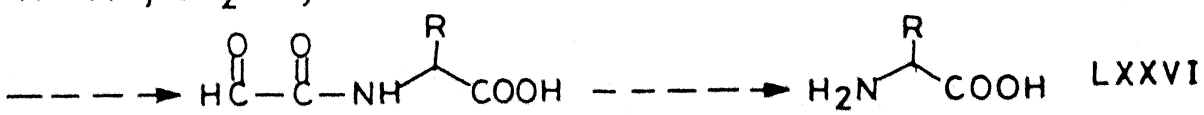


LXXIII LXXIV



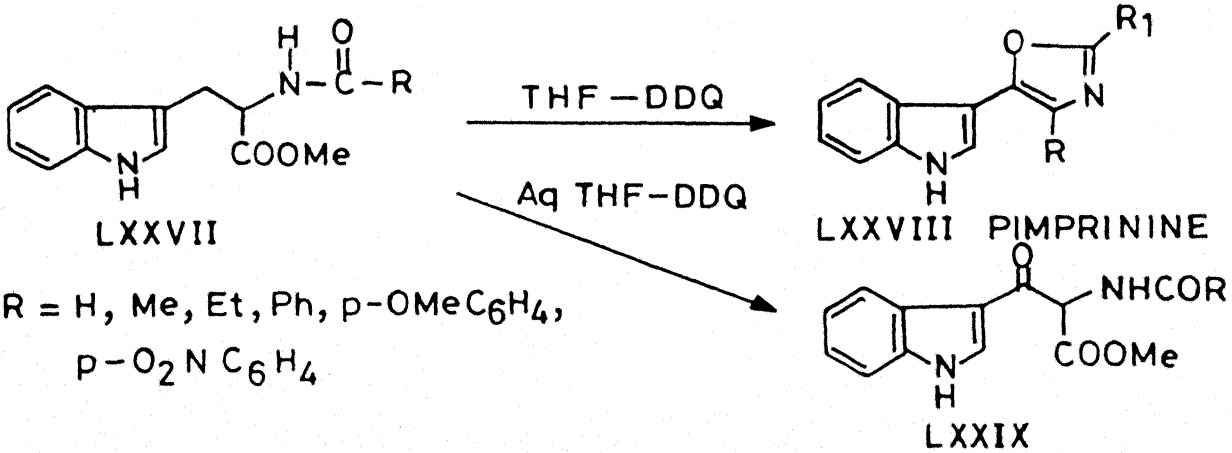
LXXV

R = Me, CH_2Ph, ^iBu



LXXVI

CHART B-30



LXXVII LXXVIII PIMPRINE LXXIX

R = H, Me, Et, Ph, p-$OMeC_6H_4$, p-$O_2NC_6H_4$

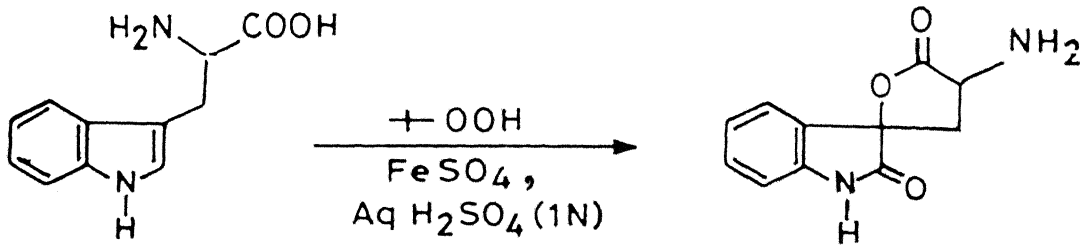
The LXXVII → LXXVIII transformation can be effected directly under non-aqueous conditions (CHART B.30)[30].

Many oxidations of the indole nucleus of tryptophan are initiated via acceptance of the oxidizing agent across the highly reactive indole 2,3 bond. Such adducts can fragment in many ways and an unusual pathway is that shown in CHART B.31 where the tryptophan carboxyl participation leads to the fragmentation of the initially formed ^{t}butylhydroperoxide adduct giving rise to the oxindole derivative LXXX (CHART B.31)[31].

The tryptophan → oxindolylalanine change can be brought about with exceptional ease using DMSO as the oxygen transfer reagent. This reaction, carried out in presence of conc. HCl, involves the protonation of the indole 3-position followed by acceptance of DMSO and collapse. The versatility of this oxygen transfer is attested by the fact that tryptophan residues in a variety of peptide substrates, such as Phe-Val-Gln-Trp-Leu-OH, valine gramicidin - A, ethanol amide and hen egg - white lysozyme are completely transformed to oxindolyl alanine residues without apparently affecting other susceptible residues such as cysteine, tyrosine and histidine, the lone exception being methionine (CHART B.32)[32].

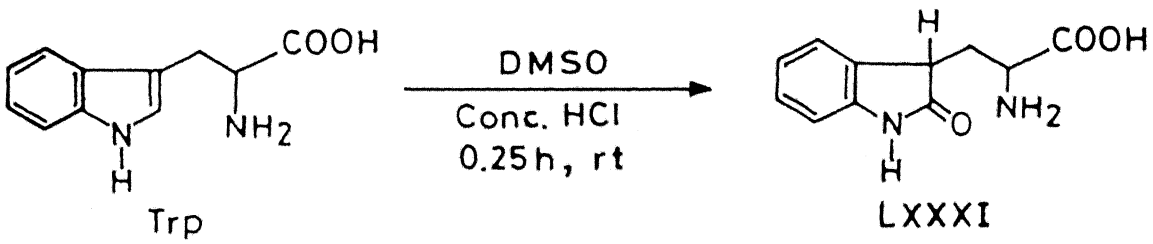
The difficulties pertaining to site specific side chain transformations chemically without affecting other equivalent and equally susceptible residues have deterred the deployment of chemical methodologies towards site specific enzyme modification. That such objectives can be realized is demonstrated by the sequential oxidation of the tryptophan residues in E.Coli B : thioredoxin. Thioredoxin, possessing 108 amino acid residues, carries tryptophan residues at 28 and 31 locations. Reaction of this with 3 equivalents of NBS at pH 4 leads

CHART B-31

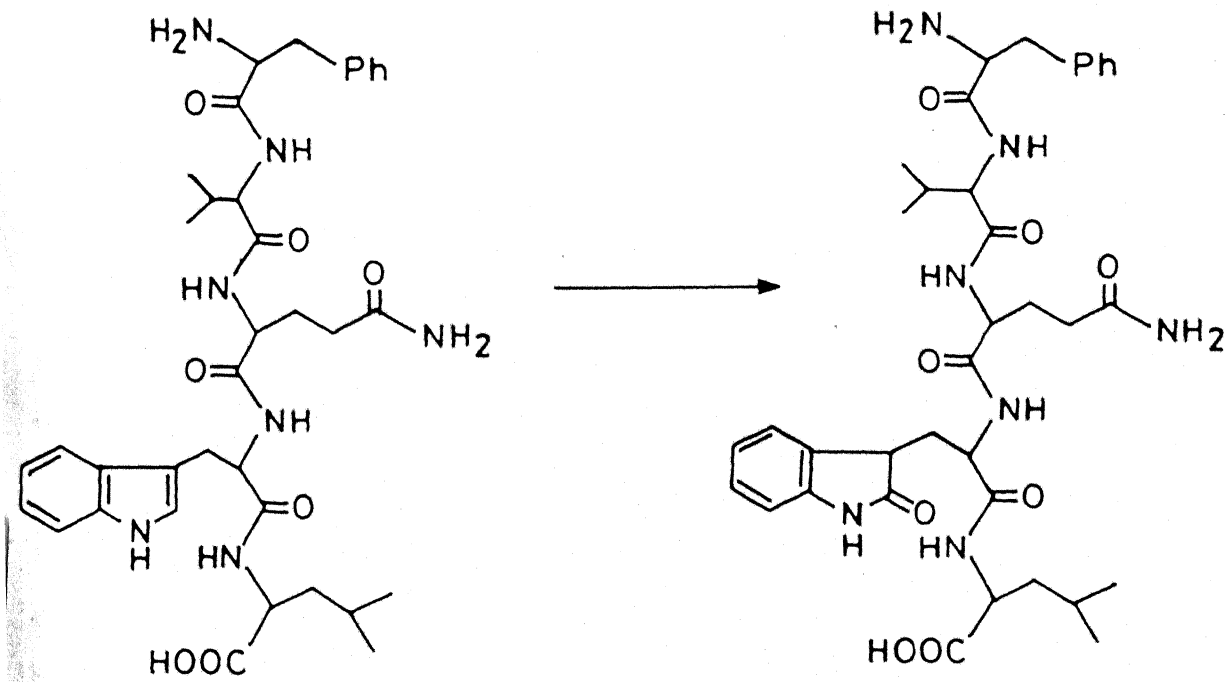


Trp LXXX

CHART B-32



Trp LXXXI



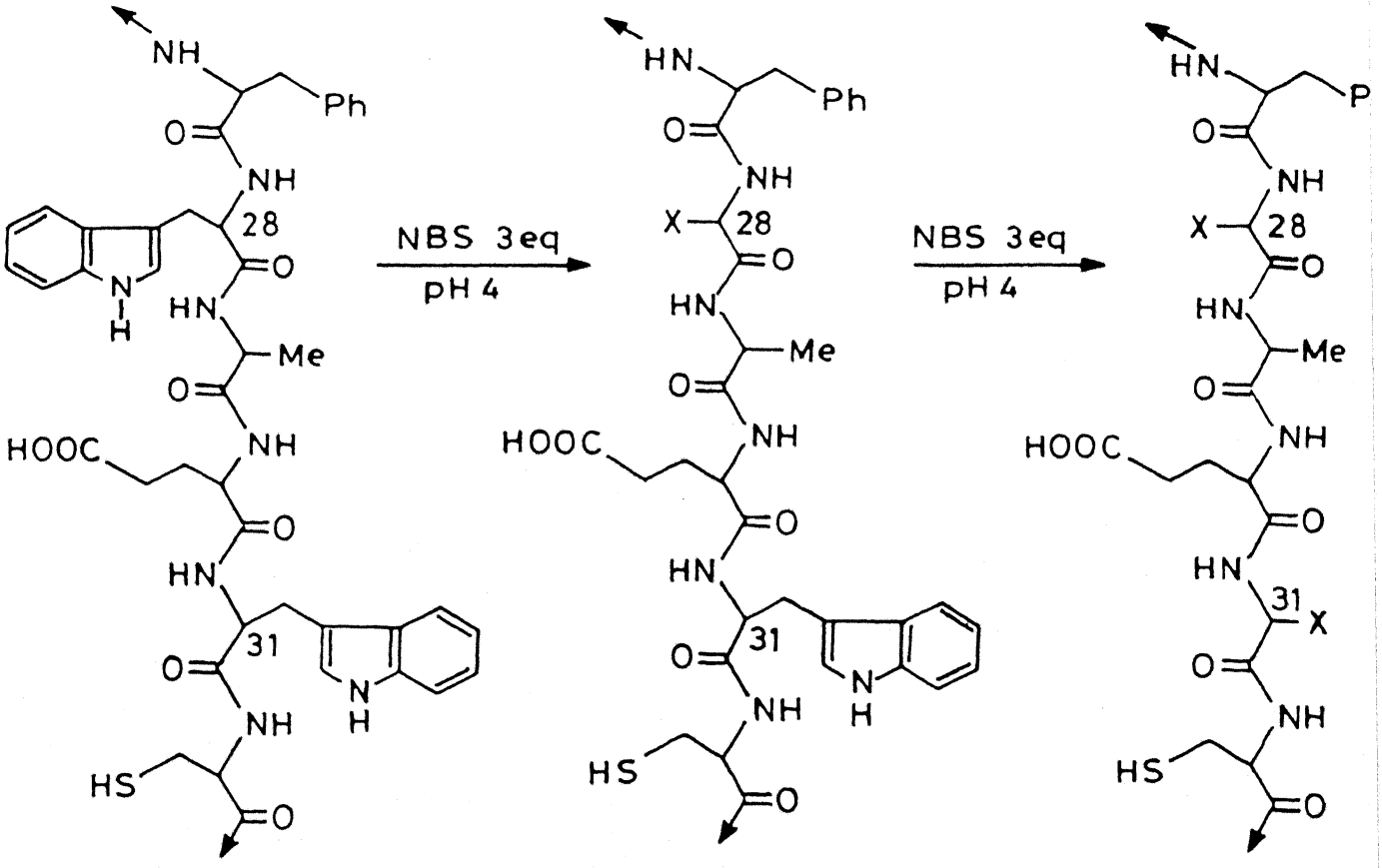
H-Phe-Val-Gln-Trp-Leu-OH LXXXII

to the specific transformation of the 28-tryptophan to an oxindolyl residue and the resulting LXXXIII retains the thioredoxin activity, demonstrating thereby the non-contribution of Trp(28) towards enzyme activity. Treatment of LXXXIII with a further 3 equivalents of NBS leads to LXXXIV where both the tryptophan residues are altered. This compound was found inactive. The fact that the E.Coli B: thioredoxin → LXXXIII → LXXXIV changes can be performed without affecting other residues such as cysteine is significant (CHART B.33)[33].

The paucity of tryptophan residues in peptides has been referred to earlier. Consequently, the reliable methodology illustrated in CHART B.34 to affix this residue to peptide side chains assumes great signficance. The methodology involves the generation of the tricyclic product LXXXV which collapses to the electrophilic intermediate LXXXVI. Addition of the cysteine SH to LXXXVI followed by aromatisation results in the attachment of the tryptophan unit. The versatility of this method is demonstrated by the fact that Trp residues can be attached to all the 8 cysteine side chains present in reduced ribonuclease (CHART B.34)[34].

N^{α}-Pthaloyl N-acetyl tryptophan can be expected to react largely as a π-bonded system. Consequently, its transformation to LXXXVIII involving the cleavage of the 2.3 π-bond with chromic acid, is not surprising. However, the formation of LXXXIX as a minor product, which must involve the reaction of the indole unit as an eneamine is noteworthy. This property is further illustrated in the formation of XC with NBS - ^{t}Bu-OH involving the 3-bromo compound as an intermediate. Compound XC, on further bromination with NBS at the highly reactive 3-indolyl position, undergoes intramolecular cyclization leading to the spiro compound LXXXIX which is the N-protected derivative of LXXX arising from reaction of tryptophan with ^{t}butylhydroperoxide-$FeSO_4$(CHART B.35)[35].

CHART B-33



E. Coli B: Thioredoxin

LXXXIII

LXXXIV

CHART B-34

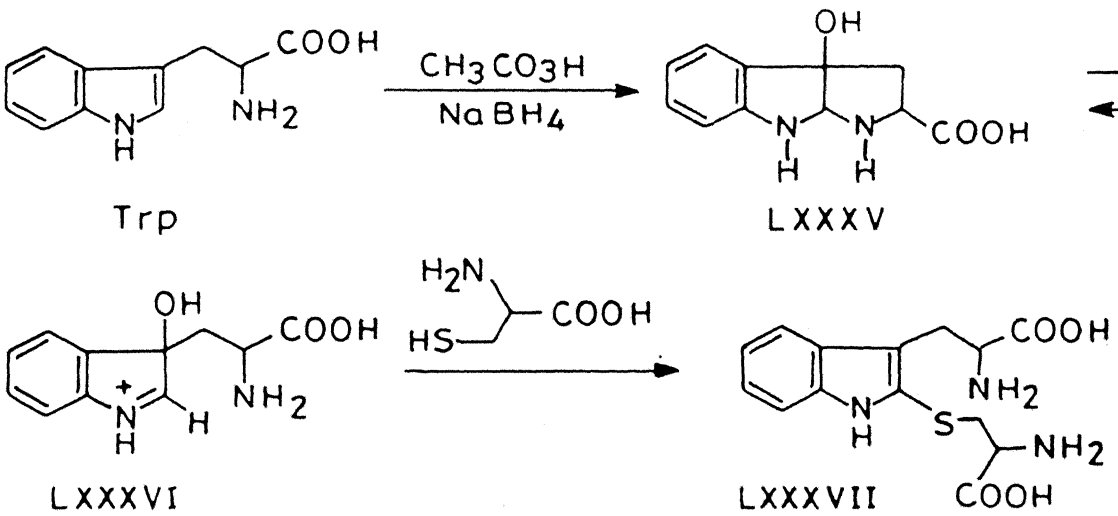
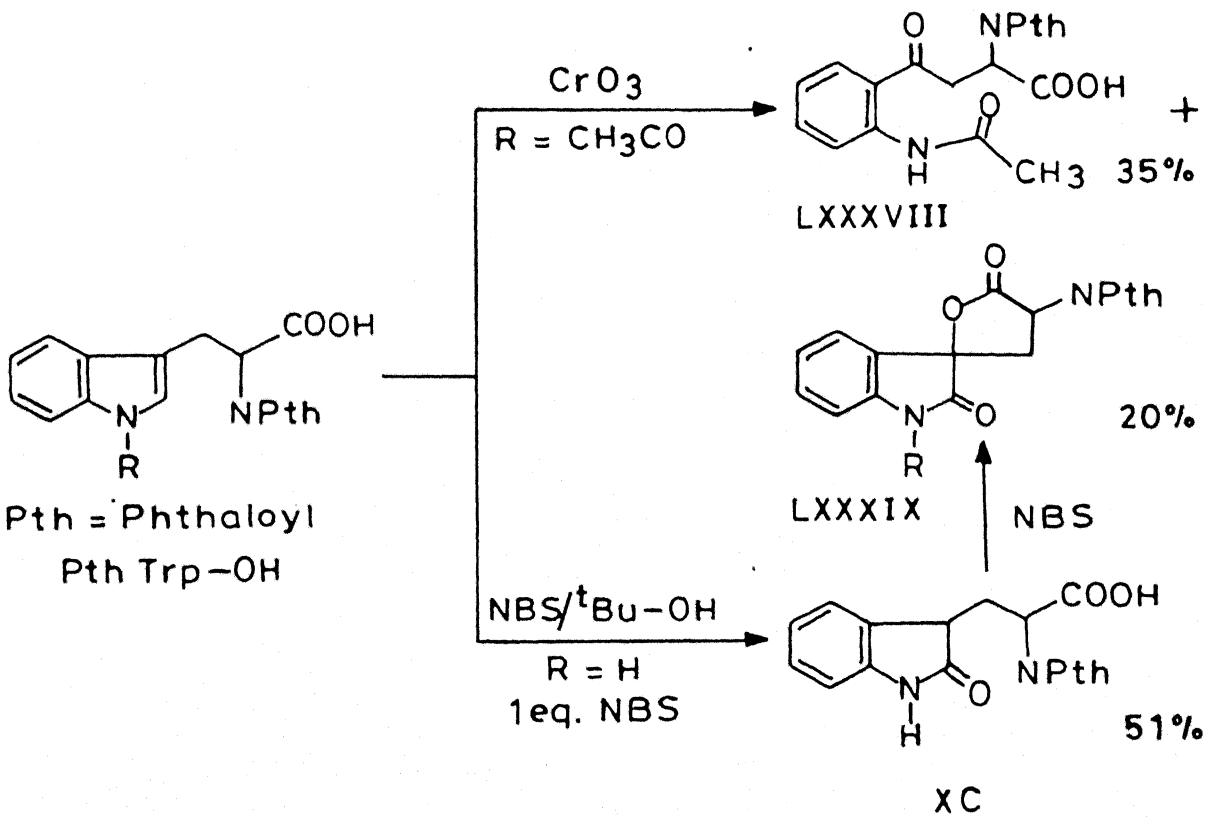


CHART B-35



The rupture of the 2,3 - indolyl π -bond of tryptophan can be cleanly effected with ozone leading to, in the case of BzTrpOMe, the formyl kynurenine XCII in about 50% yield[36]. Such a cleavage can be carried out in over 70% yields using the ozone equivalent 4-^{t}butyl iodoxybenzene. This transformation can be rationalized on the basis of adduct XCI followed by oxygen transfer giving rise to XCII and 4-^{t}butyl iodobenzene (CHART B.36)[10].

Since the early days of developments in protein chemistry, the photochemistry of tryptophan and its derivatives have attracted particular attention for several reasons. The beginnings of this interest can be attributed to the identification of the indole unit as a chromophore, thus enabling the wide use of this property in studies related to the mechanisms of enzyme action. Superimposed on this is the finding that in numerous situations involving protein damage, tryptophan photochemistry plays a pivotal role. Developments pertaining to in vitro tryptophan photochemistry over the past three decades have enabled the understanding of a multitude of such reactions on the basis of a few common pathways.

The reaction of the N,C-protected tryptophan XCIII with singlet oxygen generated by Rose Bengal sensitized photolysis leads to the hydroperoxy intermediate XCIV. Further transformations of this key intermediate can be modulated by the media. In MeOH containing pyridine, XCIV undergoes intramolecular cyclization to XCV which, in turn, can either undergo reduction to XCVI, or rupture giving rise to either kynurenine XCVII, or formylkynurenine XCVIII. In aqueous media, the hydrolytic fragmentation is the major pathway giving rise to XCVIII. In alkaline media, the conjugate base of XCIV initiates intramolecular addition to dioxetane C which collapses to the N-formylkynurenine XCVIII (CHART B.37)[36].

Hydroperoxide intermediates similar to XCV also arise on reaction of tryptophan with singlet oxygen generated by sensitized irradiation. In non-aqueous media like methanol, such an intermediate undergoes a Baeyer - Villiger type of rearrangement leading to oxazines (CI) (CHART B.38)[37].

The tricyclic hydroperoxide intermediate CII, arising from the reaction of tryptophan with singlet oxygen in aqueous-alcoholic media, exists in equilibrium with the open intermediate CIII. Compound CII can be readily reduced with dimethyl sulfide to LXXXV (CHART B.39)[38].

The open hydroperoxy intermediate CIII has been shown to fragment via two pathways. Addition of water to CIV followed by fragmentation gives N-formylkynurenine CV which also undergoes partial hydrolysis to CVI. On the other hand, a Baeyer - Villiger type of rearrangement of CIII followed by ring opening and cleavage leads to o-aminophenol, CIX (CHART B.40)[39].

The tricyclic alcohol LXXXV also exists in equilibrium with the open form LXXXVI. The latter, on addition of water and aromatization, easily gives rise to the oxindolylalanine LXXXI. This compound, like LXXXVI (CHART B.34), can be used to attach the tryptophan residue to the sulfhydryl unit of cysteine (CHART B.41)[40].

The photochemistry of N-formylkynurenine (CV) is interesting. Aqueous solutions of CV, on photolysis, yield N-formylanthranilic acid (CXIII) and 4-hydroxy-quinoline (CXVI). The formation of CXIII can be understood on the basis of hydrolysis of intermediate CXI arising from oxidation of CV. It appears that the formation of CXVI does not require either oxidation, or photolysis. On the other hand, cyclization of CV to CXIV - already known - followed by proton acceptance and fragmentation remarkably similar to that presented in CHART B.29

CHART B-36

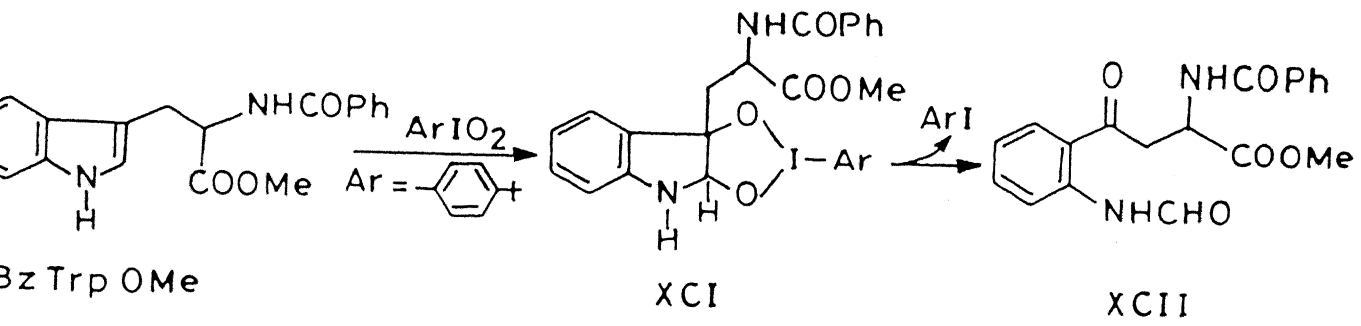


CHART B-37

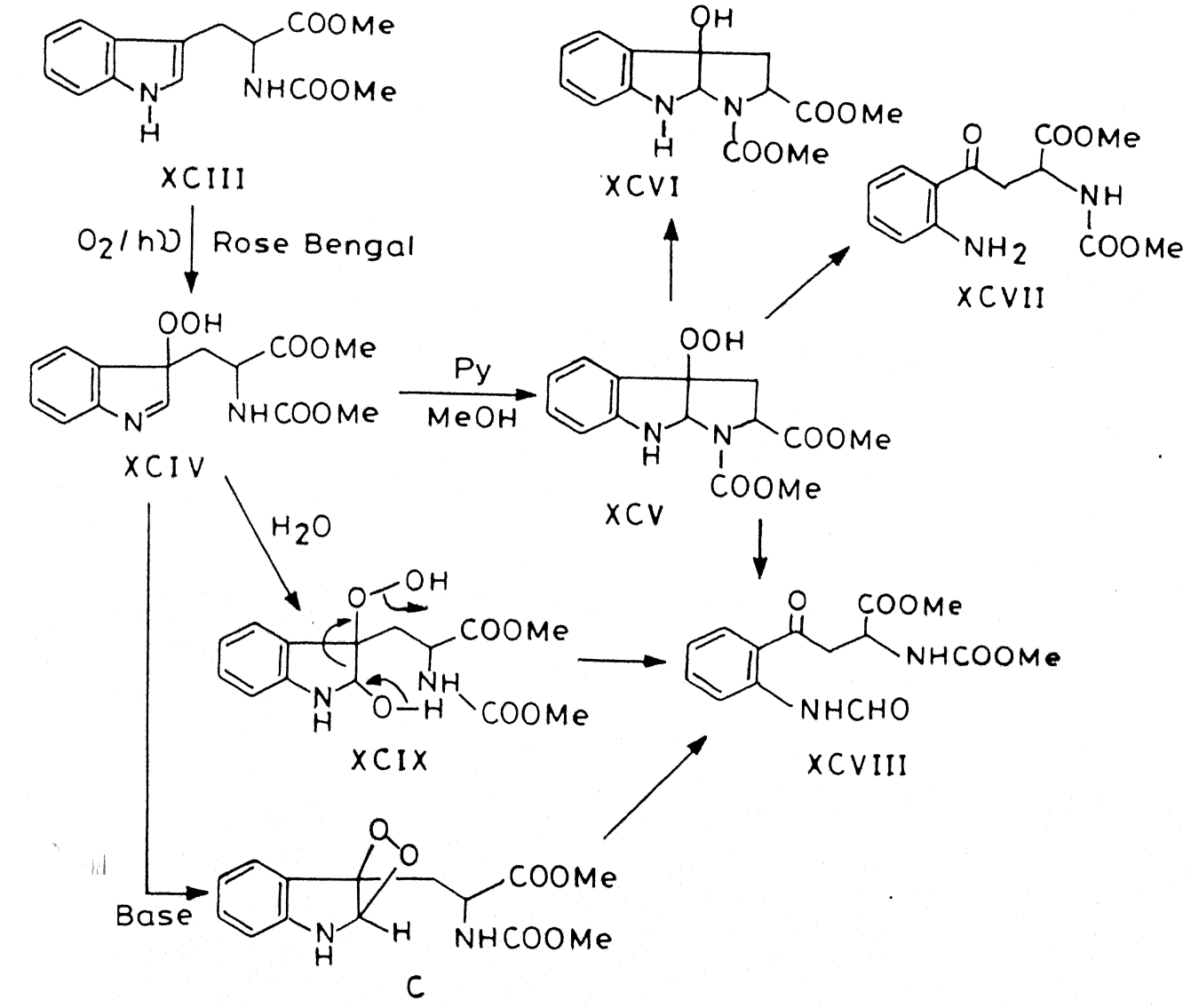


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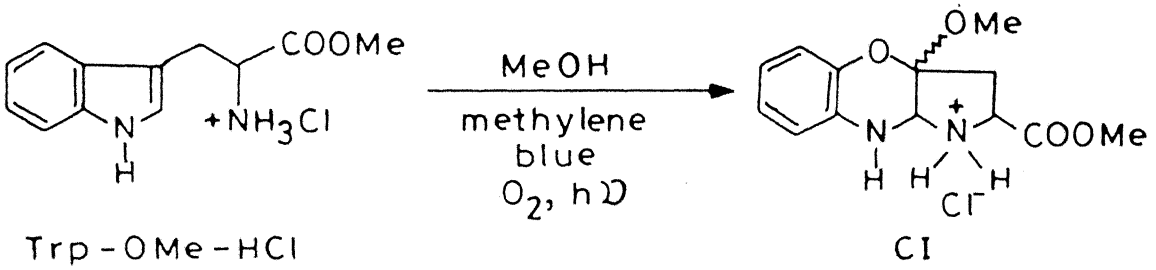
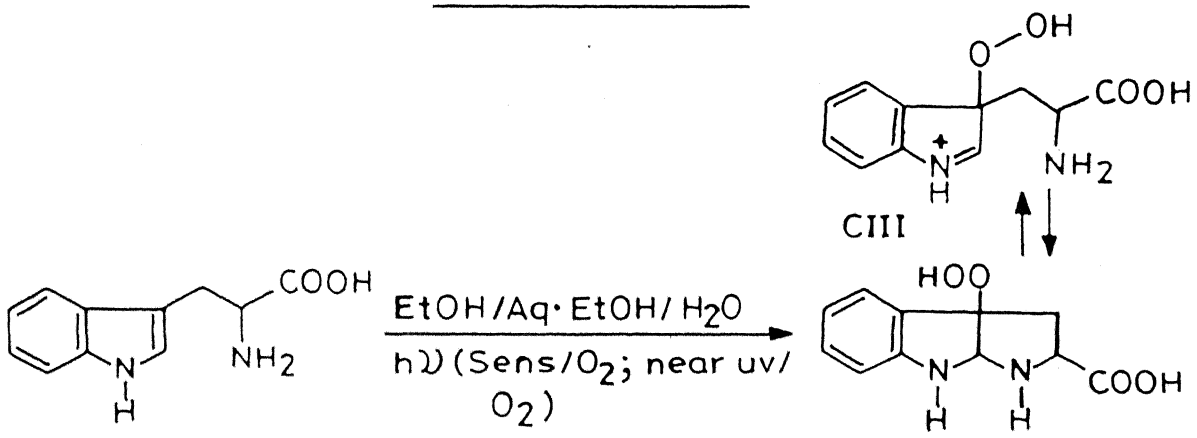
CHART B-38



Trp-OMe-HCl

CI

CHART B-39



Trp

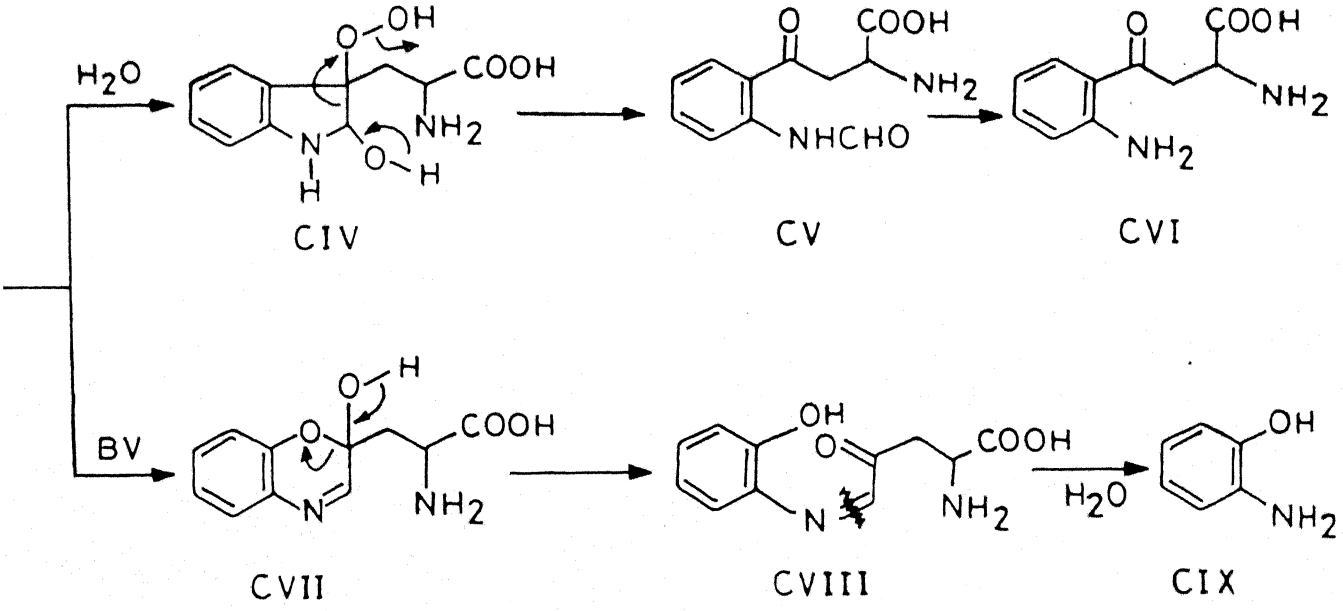
CIII

CII

Me_2S

LXXXV

CHART B-40



CIV CV CVI

CVII CVIII CIX

CHART B-41

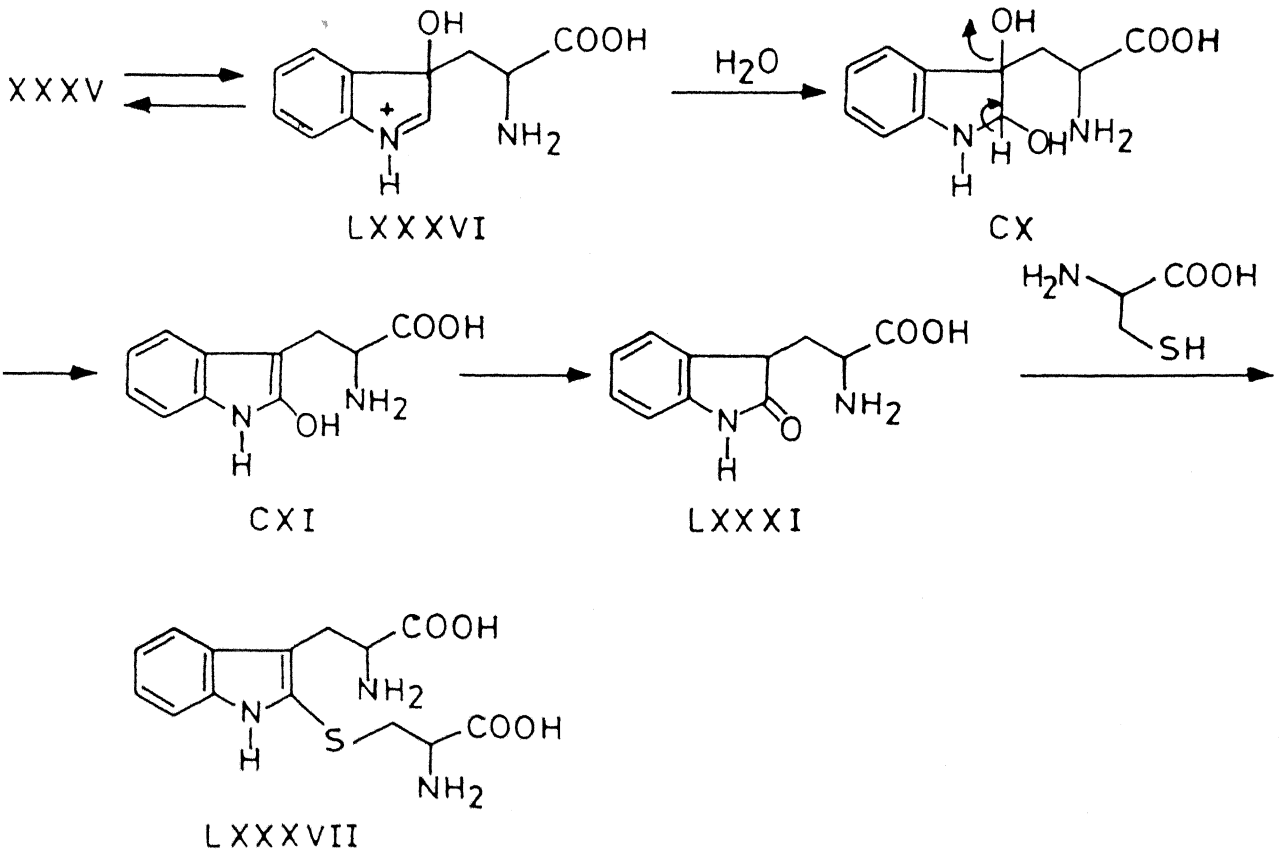
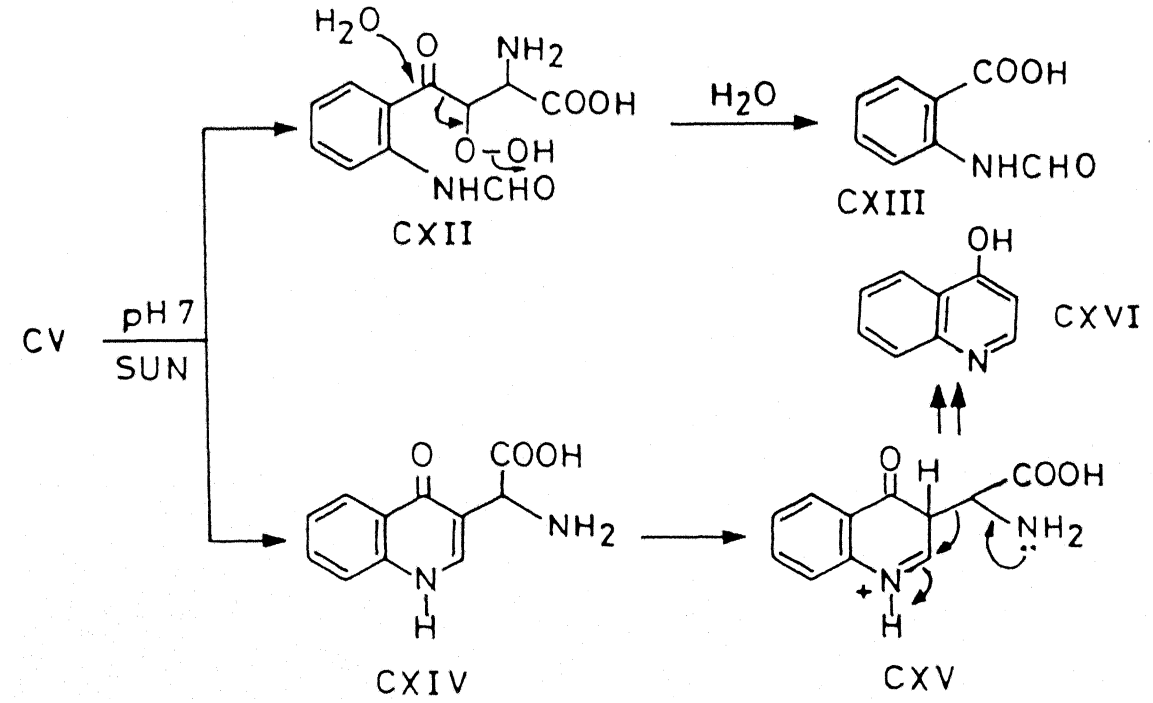


CHART B-42



would lead to CVI (CHART B.42)[41].

The reaction of tryptophan with photochemically generated singlet oxygen has been studied as a function of the pH of the media. In the pH range of 3.6 - 7.1, the tricyclic hydroperoxide CII is the main product. Interestingly, at higher ranges of pH (7.7 - 8.4), the oxidation of the aromatic ring takes place leading to the 4-hydroxy kynurenine CXX (CHART B.43)[36].

The solid state interaction of AcTrpOMe with singlet oxygen generated via microwave irradiation leads to kynurenine CXXI in an unexceptional manner. However, the reaction of AcTrpOMe with NBS is reported to produce the exceptionally strained tricyclic system CXXII which, with singlet oxygen, opens up to 7-membered ring containing compound CXXIII (CHART B.44)[37].

The photochemistry (> 295 nm) of Ala-Trp-Val, Val-Trp-Ala, Ala-Ala-Trp-Val and Ala-Gly-Trp-Leu have been investigated. In all these cases, a substantial amount of products had molecular weights 2-4 times that of the starting material indicating cross-linking. Using labelled substrates, it has been possible to show that the tryptophan residue in Ala-Gly-Trp-Leu is transformed to a mixture of N-formylkynurenine and oxindole. These results have a bearing on protein damage arising from photo-oxidation of tryptophan residues (CHART B.45)[42].

TYROSINE :

The presence of the phenolic unit in tyrosine endows it with diverse possibilities, thus making it one of the key elements in the biosynthesis of a large number of natural products. As anticipated, the oxidation of tyrosine can be brought about rather easily. Thus, BzTyrOR with Cr^{VI}- AcOH gives rise to

CHART B-43

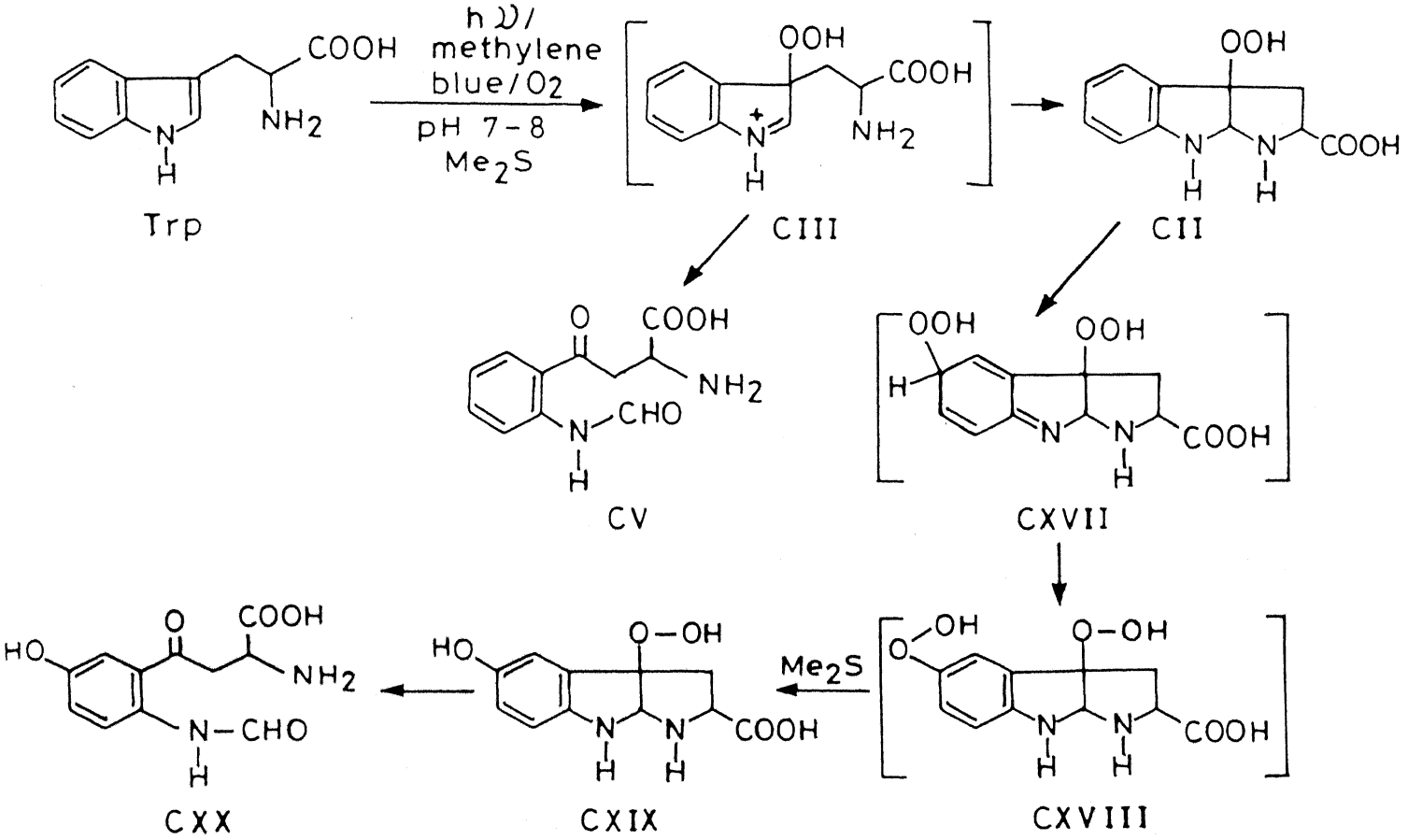


CHART B-44

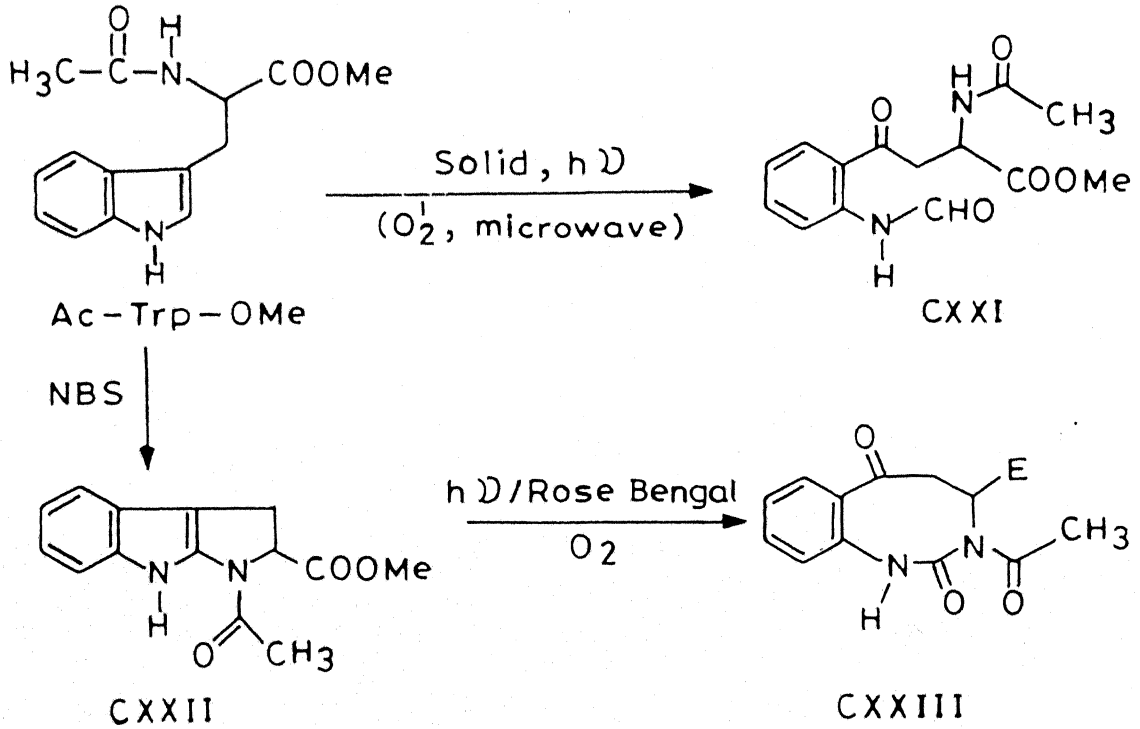


CHART B-45

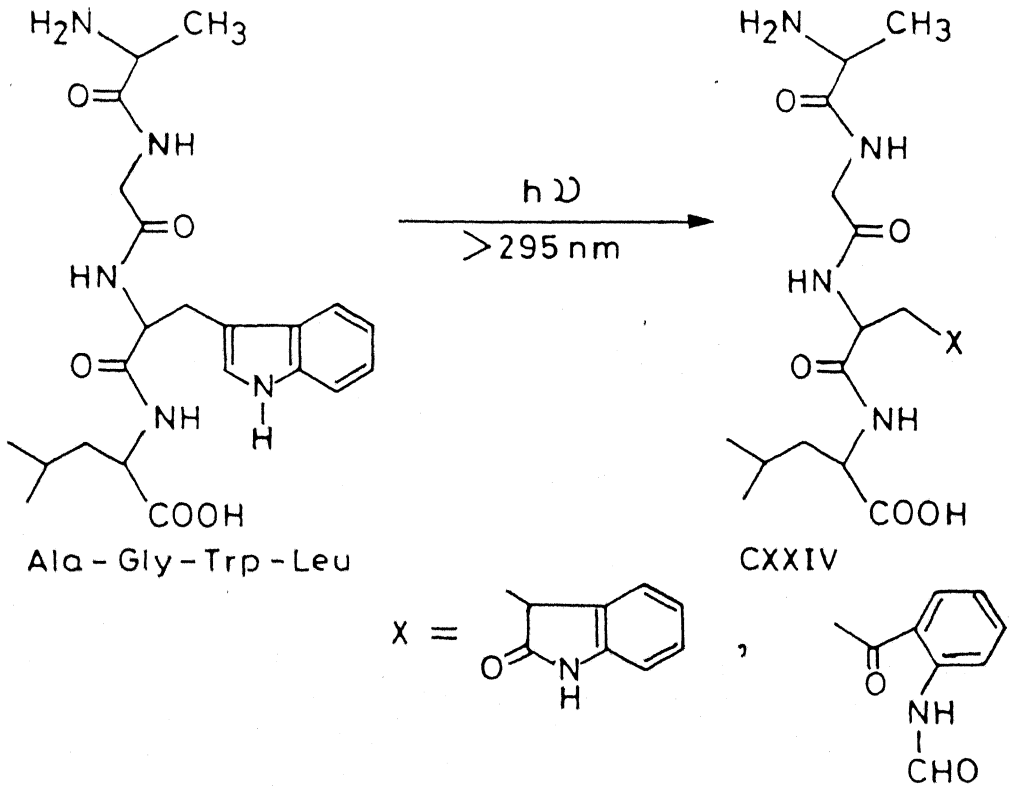

H2N
CH3
O
NH
O
HN
O
NH
N
H
COOH
hν
>295 nm
X
Ala - Gly - Trp - Leu
CXXIV
X =
O
N
H
,
O
NH
CHO

CHART B-46

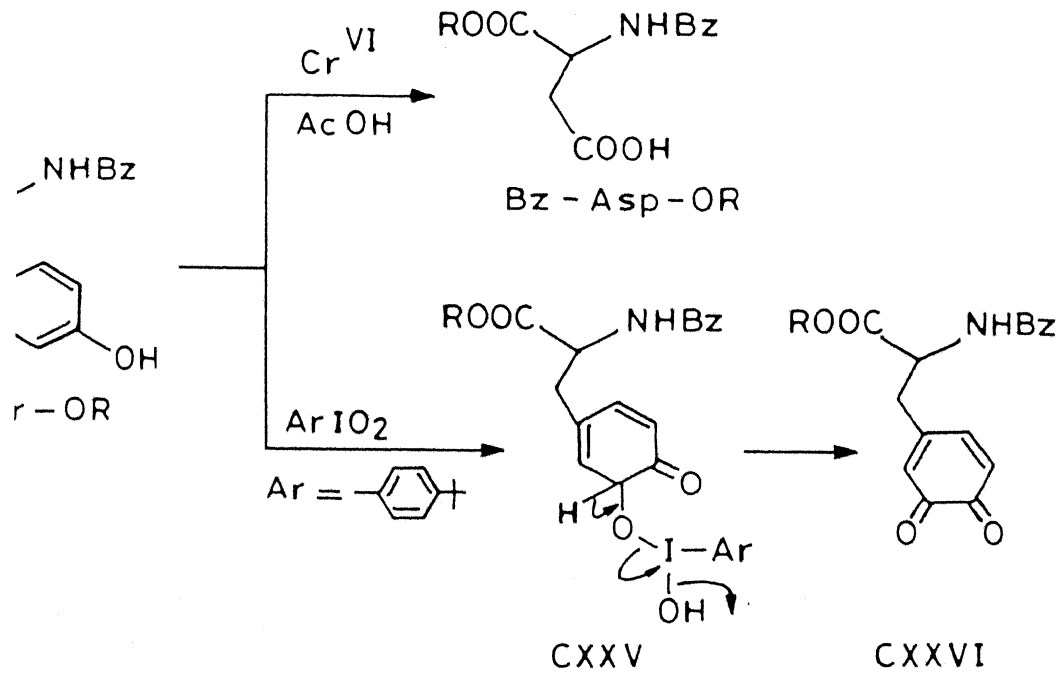
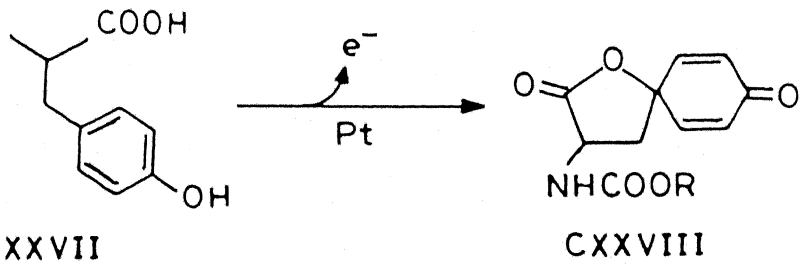


CHART B-47



the corresponding aspartic acid. The reaction of the same compound with 4-^{t}butyl iodoxybenzene leads to the expected quinone CXXVI (CHART B.46)[43,10].

The electrochemical oxidation of the N-protected tyrosine CXXVII yields the interesting spiro system CXXVIII (CHART B.47)[44].

The preceeding discussion, hopefully, has brought out a wealth of information having a bearing on the chemical modification of peptides and proteins. Above all, it confirms the notion that to a large extent, the strategies and tools available in the domain of organic chemistry can be transplanted to amino acids and peptides. The examples cited above include, marginal modifications such as indoles to oxindoles and methionine to methionine S-oxide, drastic changes as illustrated with the conversion of tyrosine to aspartic acid on the one hand and aspartic acid to alanine on the other, as well as changes that could drastically alter the tertiary structures of proteins exemplified by the proline to glutamic acid conversions.

standing of the need and consequences of such mutations has become possible.

During the last decade, a variety of methodologies and techniques have made it possible to correlate the 3-dimensional structure of a functional system to the reaction which it can specifically bring about in living cells. These have shown, as never before, the infinite care with which the individual amino acid residues that form even the most complex of enzymes are put together. Further, it has been demonstrated that apart from these amino acids that are involved in the enzyme active sites, a very large number of other residues are equally important and contribute to the overall efficiency of the functional system in a significant manner[46]. It is now recognized that mutations have a bearing on changing cellular environments, although in most cases these are difficult to discern. Additionally, although a point mutation in the information system could generate a range of changed proteins, over a period of time, only a selected few survive. The domain pertaining to the study of functional systems with particular reference to site directed side chain alteration has multi-dimensional relevance. The understanding of such studies would lead to the explanation of the rationale behind alteration of each one of these side chain residues that are incorporated on the one hand, and would provide incisive information pertaining to the reactions mediated by enzymes, particularly those associated with genetic disfunction, on the other.

The problems associated with the replacement of a single side chain residue in an enzyme are quite formidable. In broad terms, whilst genetic engineering has as its basis the study of consequences of the arrangement of four code bases in the information system, that relating to protein engineering calls for a study of compounds arising from the permutation of 20 coded amino acids !

Nevertheless, recent endeavours have shown that this can be accomplished. Three methodologies are available to effect such changes on the protein backbone. The most reliable strategy is via operation at the information level, particularly by a technique named "cassette mutagenesis" which enables the insertion of small segments of appropriate nucleic acids containing the required codon for the desired site alteration, followed by normal genetic engineering techniques leading to the isolation of the altered protein[47]. In spite of the fact that this method is very powerful, it necessarily entails complicated methodologies and is generally not well suited for the preparation of quantities of products. The replacement of specific amino acid residues by enzymatic methods which could be illustrated by the transformation of porcine insulin into human insulin involving the replacement of a terminal alanine with a threonine[48] could be useful under very selective circumstances.

The development of chemical methodologies to effect alteration at the enzyme level would necessarily entail less energy compared to the current methods, where the starting point for such alterations is at the higher, genome level. In principle, the vast array of strategies and tools available in the domain of chemistry could be deployed to alter proteins in the desired manner. However, such approaches have hitherto not found much favour, possibly because of apprehensions regarding bringing about highly precise changes in extremely complex molecular systems such as those present even in relatively simple polypeptides.

The work presented in this section endeavours to illustrate that chemoselective methodologies can be developed to effect side chain alterations in peptides and that the result obtained in the present work could be transplanted to even more complex functional systems.

The genetic code is presented in CHART C.I . Assuming that there is a rationale in the alteration of protein structure, either as a function of evolution or other genetic events, a broad appreciation of the correlation of the triplet codes with the amino acids they refer to is important. A noteworthy feature of the genetic code is that the degeneracy of the code is non-random and a broad pattern of organization can be discerned. For example, the 5 pairs of amino acids, namely, Phe - Leu, Ser - Arg, His - Gln, Asn - Lys, and Asp - Glu share a common doublet, appropriating, uniformly, a pyrimidine partner for one and a purine for the other. The possible significance of this emerges from a study of the mutation patterns of a large number of unrelated proteins. As shown in CHART C.II, a single point mutation in the genome could generate a range of amino acids[49]. The analyses presented in CHART C.III clearly indicate that some mutated functional systems are preserved over the others[50]. Of particular relevance is that in practically every case, a point mutation tends to favour the partner alluded to above over others.

In view of the nearly complete lack of information, expertise and methodologies in the literature pertaining to bring about chemoselective protein modification, it was considered advantageous to define the initial objectives at the outset. These were governed by two guiding factors. The transformation of a particular side chain residue to another that has latent possibilities for further elaboration appeared as an attractive objective. This is illustrated in CHART C.IV . The second guideline was related to the strategy wherein the methodology that is to be developed should have a broad base, in the sense that it should have the capability of affecting as many side chains of the 20 coded amino acids as possible. It was felt that from such a broad base, by further

CHART C-I

First position (5' end)	Second position				Third position (3' end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Term	Term	A
	Leu	Ser	Term	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

CHART C - II

Amino Acid Replacements that May Occur as a Result of Single Base Changes in the Genetic Code

Amino acid	Replacement
TRP	ARG, GLY, SER, LEU, CYS
GLN	ARG, PRO, LEU, LYS, GLU, HIS
PHE	LEU, ILE, VAL, SER, TYR, CYS
ASN	SER, THR, ILE, TYR, HIS, ASP, LYS
PRO	HIS, ARG, LEU, GLN, THR, ALA, SER
ASP	GLY, ALA, VAL, ASN, HIS, TYR, GLU
MET	THR, VAL, LYS, LEU, ARG, ILE
LYS	ARG, THR, ILE, MET, GLN, GLU, ASN
CYS	TYR, SER, PHE, ARG, GLY, TRP
HIS	TYR, GLN, ASN, ASP, ARG, PRO, LEU
ALA	VAL, ASP, GLY, GLU, THR, PRO, SER
GLU	GLY, ALA, VAL, ASP, LYS, GLN
VAL	ALA, MET, ILE, LEU, GLY, GLU, ASP, PHE
TYR	CYS, SER, PHE, HIS, ASN, ASP
THR	ILE, ASN, MET, LYS, ARG, SER, ALA, PRO
GLY	ARG, SER, TRP, CYS, VAL, ALA, ASP, GLU
ILE	LEU, PHE, VAL, THR, ASN, LYS, SER, ARG, MET
LEU	ILE, VAL, MET, SER, TRP, PHE, PRO, HIS, GLN, ARG
SER	PHE, TYR, CYS, LEU, TRP, PRO, THR, ALA, ASN, ILE, GLY, ARG
ARG	HIS, LYS, CYS, TRP, SER, GLY, LEU, PRO, GLN, THR, MET

CHART C-III

	Gly	Ala	Val	Leu	Ile	Met	Cys	Ser	Thr	Asn	Gln	Asp	Glu	Lys	Arg	His	Phe	Tyr	Trp	Pro
Gly		44	38				16	16				21	24		43				13	
Ala	58		41					35	39			23	26							36
Val	10	37		48	18	12						20	23				16			
Leu	2	10	30		16	21		13			16				22	11	41		9	26
Ile		7	66	25		25		15	17	18							16			
Met	1	3	8	21	6				11					19	7					
Cys	1	3	3		2			24							10		12	15	16	
Ser	45	77	4	3	2	2	12		41	15					30		13	15	8	24
Thr	5	59	19	5	13	3	1	70		17				24	12					28
Asn	16	11	1	4	4			43	17			19		49		13		18		
Gln	3	9	3	8	1	2		5	4	5			23	24	13	26				15
Asp	16	15	2		1			10	6	53	8		49			15		20		
Glu	11	27	4	2	4	1		9	3	9	42	83		30						
Lys	6	6	2	4	4	9		17	20	32	15		10		20					
Arg	1	3	2	2	3	2	1	14	2	2	12	9		48		8			13	20
His	1	2	3	4			1	3	1	23	24	4	2	2	10			13		10
Phe	2	2	1	17	9	2		4	1	1					1	2		16		
Tyr		2	2	2	1		3	2	2	4			1	1		4	26			
Trp				1				2							3		1	1		
Pro	5	35	5	4	1		1	27	7	3	9	1	4	4	7	5	1			

Observed Number of Replacements

Expected Number of Random Mutational Events

CHART C-IV

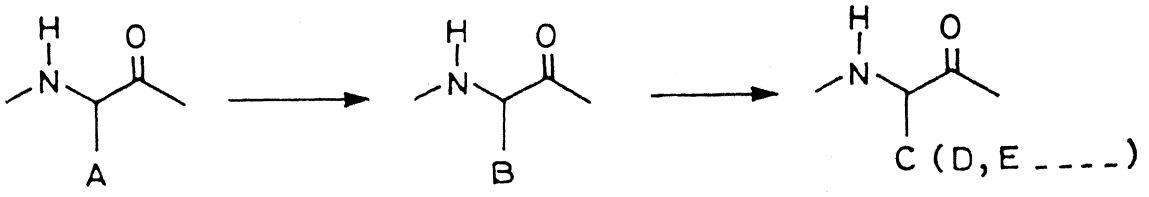


CHART C-V

PHE	SER	TYR	CYS
LEU		TERM	TERM
			TRP
LEU	PRO	HIS	ARG
		GLN	
ILE	THR	ASN	SER
MET		LYS	ARG
VAL	ALA	ASP	GLY
		GLU	

: SUCEPTIBLE TO OXIDIZING AGENTS

: INERT TO OXIDIZING AGENT

developments, operating on diverse factors such as the duration of the reaction, the amounts of the appropriate reagents, and the nature of the media, it should be possible, by performing a series of relevant experiments, to achieve increasing selectivity, enabling the targeting to a selected residue amongst many, which, in principle, are susceptible.

Of the 20 coded amino acids, as many as 13, in principle, are susceptible to oxidizing agents. This is illustrated in CHART C.V . Consequently, the oxidative methodology became the logical starting point for the amino acid side chain alteration studies. Further, as could be seen from CHART C.V, the side chains offer a range of oxidizing profiles, from the most difficult phenylalanine to the most readily oxidizable methionine. It was felt that the only reagent that could perhaps bring about the oxidation of such a range of substrates is the in situ generated Ru^{VIII} species. The present work, thus, is an account of the reaction of such amino acid side chains with in situ generated Ru^{VIII} species and culminates in the demonstration of possibilities for using this methodology for chemoselective protein alteration.

The plan of research comprised of three stages of development, namely, the transformation of N,C - protected amino acid side chains, which would be similar to that in a peptide environment, the transformations of amino acid side chains in dipeptides and higher peptides in competitive and non-competitive circumstances, and finally the demonstration of the methodologies thus developed in more complex proteins which involve chemoselective alterations. In addition, the action of Ru^{VIII} on unprotected amino acids has been investigated in order to provide mechanistic support envisaged in oxidative transformations, as well as to clarify conflicting reports present in the literature.

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In order to facilitate reading, the description of the present work is organized as shown below :

A. The preparation of protected L-amino acids and peptides which are used as starting materials. The preparation of authentic samples of envisaged products.

B. The oxidation of N,C - protected amino acids.

C. The oxidation of dipeptides and a tetrapeptide under competitive and non-competitive circumstances.

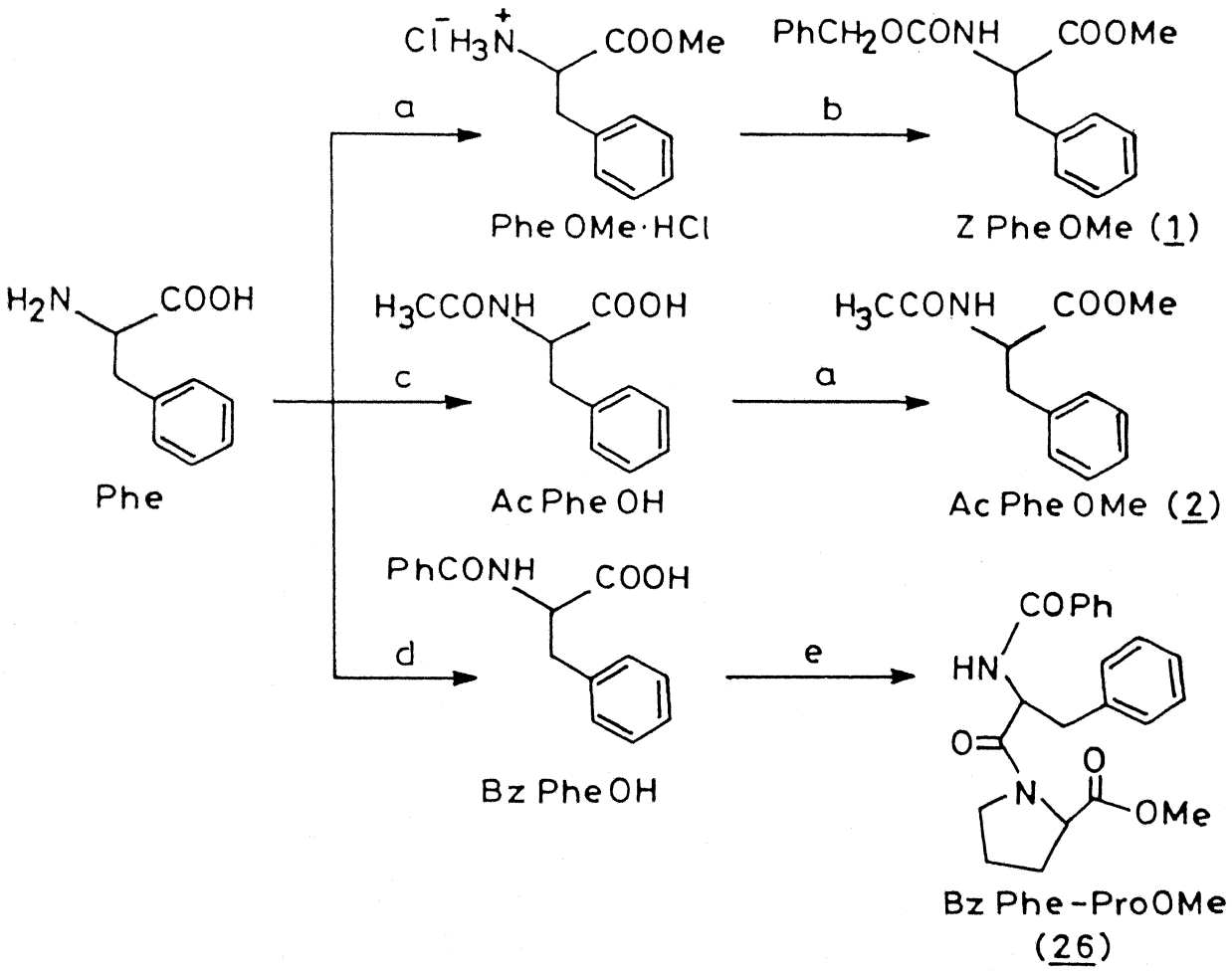
D. The chemoselective oxidation of the hydrophobic region of c- lysozyme.

E. The chemoselective oxidation of melittin.

F. Mechanistic studies on the oxidation of unprotected α -amino acids.

All oxidations have been carried out with in situ regenerated Ru^{VIII} species.

CHART C-VI



a : MeOH/$SOCl_2$; b : Cbz Cl / $NaHCO_3$; c : Ac_2O / NaOH ;
d : Bz Cl / NaOH ; e : Pro OMe / DCC / HOBt

nmr : $\delta(CDCl_3)$: 1.5 - 2.1 (br, 4H, $-CH(C\underline{H}_2)_2$), 3.05 (d, 2H, $-CHC\underline{H}_2Ph$), 3.6 (m, 5H, $-(CH_2)_2C\underline{H}_2N$,$-COOC\underline{H}_3$), 4.25 (br, 1H, $-C\underline{H}$), 5.05 (m, 1H, $-C\underline{H}$), 6.9 - 7.9 (m, 11H, $-N\underline{H}$, aromatic protons).

ms : m/z : 380 (M^+), 381 (M^++1)

The transformation of glycine to ZGlyOMe (3) is shown in CHART C.VII.

ZGlyOMe (3) :

Oil ; bp. 168 - 170°C / 1 torr.

ir : ν_{max} (neat) cm^{-1} : 3350, 1720 (ester), 1520.

nmr : $\delta(CDCl_3)$: 3.7 (s, 3H, $-COOC\underline{H}_3$), 3.9 (d, 2H, $-C\underline{H}_2COOCH_3$), 5.1 (s, 2H, $-OC\underline{H}_2Ph$), 7.25 (s, 6H, $-N\underline{H}$, aromatic protons).

BzTyrOMe (4) and BzTyr-PheOMe (22) were prepared from tyrosine (CHART C.VIII).

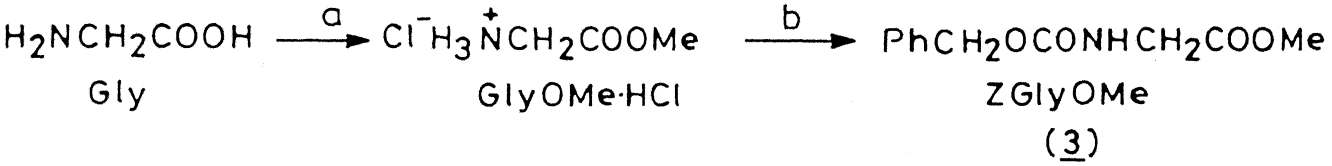
BzTyrOMe (4) :

mp. 156°C

ir : ν_{max} (KBr) cm^{-1} : 3260, 1695 (ester), 1635, 1525 (amide).

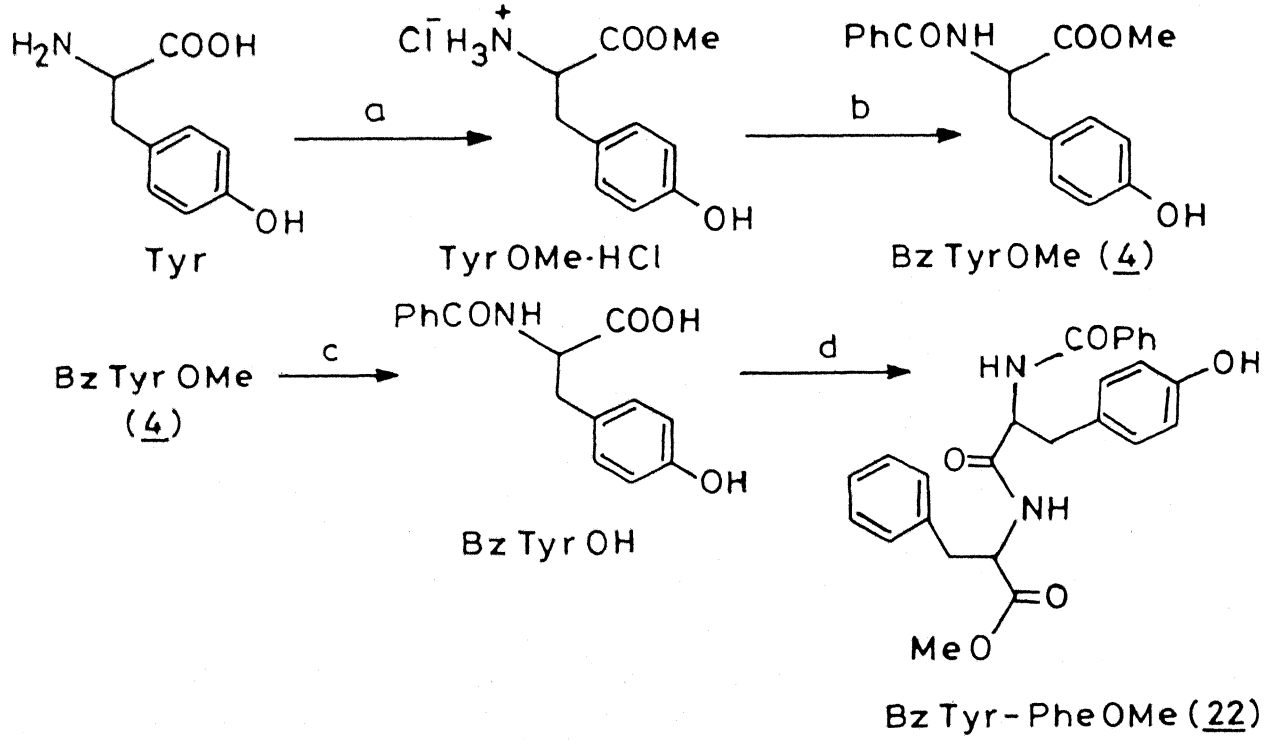
nmr : $\delta(CDCl_3$ + DMSO - $d_6)$: 3.1 (d, 2H, $-CHC\underline{H}_2$), 3.7 (s, 3H, $-COOC\underline{H}_3$), 4.85 (q, 1H, $-C\underline{H}$), 6.55 - 7.95 (m, 10H, $-N\underline{H}$, aromatic protons), 8.75 (s, 1H, $-O\underline{H}$).

CHART C-VII



a : MeOH / HCl ; b: Cbz Cl / Na HCO_3 ;

CHART C-VIII



a: MeOH / $SOCl_2$; b: BzCl / $NaHCO_3$;

c: NaOH / MeOH ; d: PheOMe / DCC / HOBt

BzTyr-PheOMe (22) :

mp. 205°C

ir : ν_{max}(KBr) cm^{-1} : 3305, 1725 (ester), 1640, 1540 (amide).

nmr : δ ($CDCl_3$ + DMSO - d_6) : 2.85 - 3.32 (m, 4H, 2 x -CHC<u>H</u>$_2$), 3.3 (s, 3H, - COOC<u>H</u>$_3$), 4.42 - 4.87 (br, 1H, -C<u>H</u>), 5.22 - 5.72 (br, 1H, -C<u>H</u>), 6.5 - 8.1 (m, 16H, -N<u>H</u>, aromatic protons).

The transformation of tryptophan to ZTrpOMe (5), BzTrpOMe (6), BzTrp-LeuOMe (23) and BzTrp-PheOMe (24) is shown in CHART C.IX.

ZTrpOMe (5) :

Thick syrup ; bp. 230-240°C / 0.5 torr.

BzTrpOMe (6) :

mp. 110 - 111°C

ir : ν_{max} (KBr) cm^{-1} : 3320 (-NH), 1720 (ester), 1620, 1575.

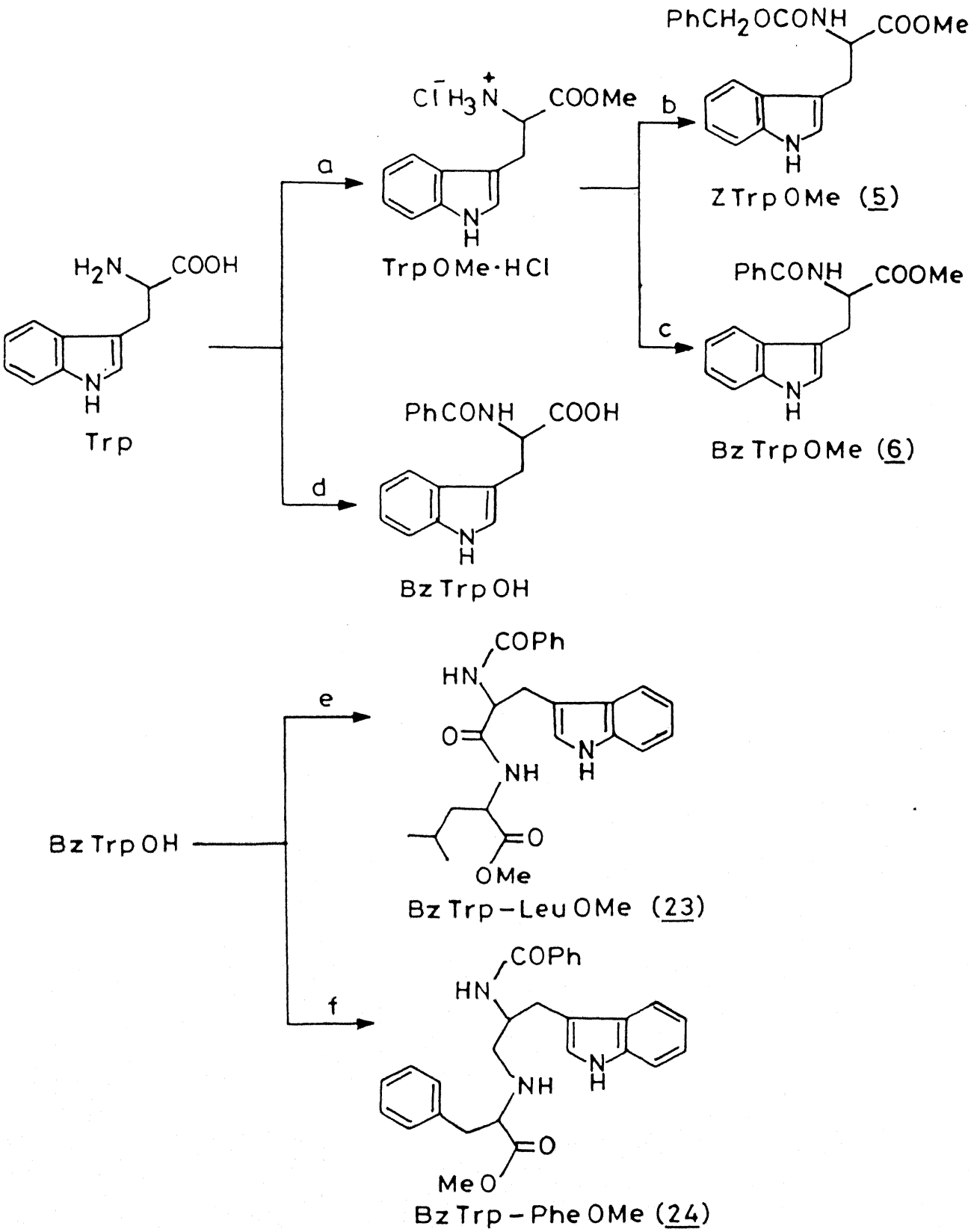
nmr : δ($CDCl_3$) : 3.4 (d, 2H, -CHC<u>H</u>$_2$), 3.65 (s, 3H, -COOC<u>H</u>$_3$), 5.1 (m, 1H, -C<u>H</u>), 6.6 - 7.9 (m, 11H, -N<u>H</u>, aromatic protons), 8.65 (br s, 1H, -N<u>H</u>).

BzTrp-LeuOMe (23) :

Gummy solid

ir : ν_{max} (neat) cm^{-1} : 3260 (-NH), 1715 (ester), 1620, 1510 (amide).

CHART C-IX



a: MeOH / $SOCl_2$; b : Cbz Cl / $NaHCO_3$; c : Bz Cl / $NaHCO_3$;
d : Bz Cl / NaOH ; e : LeuOMe / DCC / HOBt ; f : PheOMe / DCC / HOBt

nmr : $\delta(CDCl_3)$: 0.8 (br, 6H, $-CH(C\underline{H}_3)_2$), 1.45 (m, 2H, $-CHC\underline{H}_2CH$), 2.2 (br s, 1H, $-C\underline{H}(CH_3)_2$), 3.3 (d, 2H, $-CHC\underline{H}_2$), 3.6 (s, 3H, $-COOC\underline{H}_3$), 4.5 (m, 1H, -C<u>H</u>), 5.0 (m, 1H, -C<u>H</u>), 6.5 - 8.0 (m, 12H, -N<u>H</u>, aromatic protons), 8.5 (br s, 1H, -N<u>H</u>).

ms : m/z : 435 (M^+)

BzTrp-PheOMe (<u>24</u>) :

Gummy solid

ir : ν_{max} (neat) cm^{-1} : 3270 (-NH), 1720 (ester), 1625, 1510 (amide).

nmr : $\delta(CDCl_3)$: 2.8 (m, 2H, $-CHC\underline{H}_2Ph$), 3.2 (m, 2H, $-CHC\underline{H}_2$), 3.6 (s, 3H, $-COOC\underline{H}_3$), 4.9 (m, 2H, 2 x -C<u>H</u>), 6.4 - 7.8 (m, 17H, -N<u>H</u>, aromatic protons), 8.4 (br s, 1H, -N<u>H</u>).

ms : m/z : 469 (M^+), 470 (M^++1)

ZHisOMe (<u>7</u>) was prepared from histidine monohydrochloride (CHART C.X).

ZHisOMe (<u>7</u>) :

Thick syrup

ir : ν_{max} (neat) cm^{-1} : 3200 (br), 1715 (ester), 1520, 1440.

nmr : $\delta(CDCl_3)$: 3.1 (d, 2H, -CHC<u>H</u>$_2$), 3.65 (s, 3H, -COOC<u>H</u>$_3$), 4.55 (br, 1H, -C<u>H</u>), 5.05 (s, 2H, -OC<u>H</u>$_2$Ph), 6.3 (br,1H, -N<u>H</u>), 6.72 (br, 1H,-NC<u>H</u>=C), 7.3 (s, s, 5H, -CH_2C_6<u>H</u>$_5$), 7.8 (br, 1H, -NHC<u>H</u>=N-).

From methionine, BzMetOMe (<u>8</u>) and ZMetOMe (<u>9</u>) were prepared according to procedures outlined in CHART C.XI.

BzMetOMe (<u>8</u>) :

mp. 75°C

ir : ν_{max}(KBr) cm^{-1} : 3290 (-NH), 1740 (ester), 1630, 1525 (amide), 1420.

nmr : $\delta(CDCl_3)$: 2.1 (s, 3H, -SC<u>H</u>$_3$), 2.2 - 2.8 (m, 4H, -CHC<u>H</u>$_2$C<u>H</u>$_2$), 3.75 (s, 3H, -COOC<u>H</u>$_3$), 4.9 (q, 1H, -C<u>H</u>), 6.9 - 8.0 (m, 6H, -N<u>H</u>, aromatic protons).

ZMetOMe (<u>9</u>) :

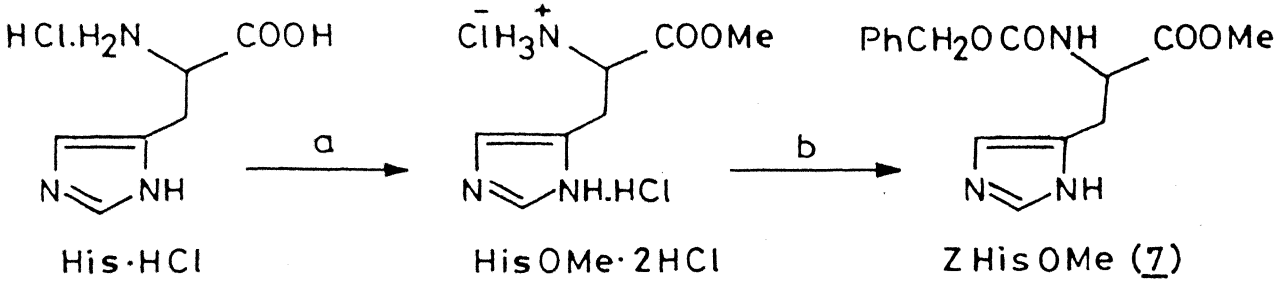
Oil

ir : ν_{max} (neat) cm^{-1} : 3315 (-NH), 1715 (ester), 1520, 1430.

nmr : $\delta(CDCl_3)$: 2.05 (s, 3H, -SC<u>H</u>$_3$), 2.15 - 2.75 (m, 4H, -CH(C<u>H</u>$_2$)$_2$), 3.7 (s, 3H, -COOC<u>H</u>$_3$), 4.35 - 4.75 (m, 1H, -C<u>H</u>), 5.05 (s, 2H, -OC<u>H</u>$_2$Ph), 5.5 (br, 1H, -N<u>H</u>), 7.25 (s, 5H, aromatic protons).

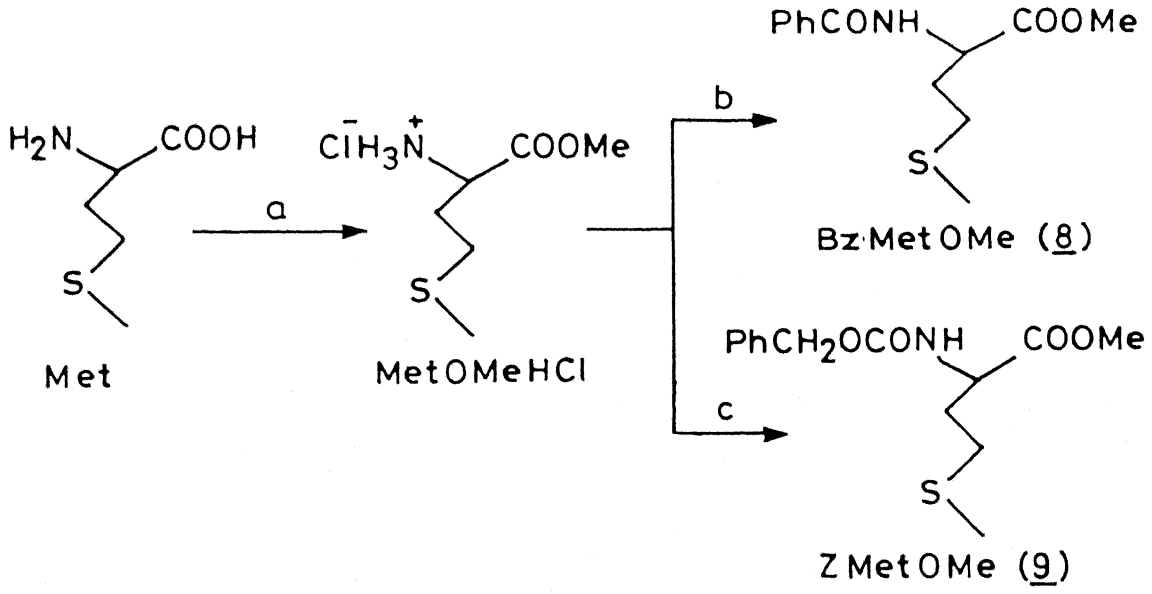
The preparation of ZCys(S - Bzl)OMe (<u>10</u>) is described in CHART C.XII.

CHART C-X



a : 1. MeOH / H_2SO_4, 2. HCl ; b : CbzCl / Et_3N

CHART C-XI



a : MeOH / HCl ; b : BzCl / $NaHCO_3$; c : CbzCl / $KHCO_3$

ZCys(S - Bzl)OMe (10) :

mp. 64 - 65°C

ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1735 (ester), 1690, 1535.

nmr : $\delta(CDCl_3)$: 2.9 (d, 2H, -CHC$\underline{H_2}$), 3.7 (s, s, 5H, -COOC$\underline{H_3}$, -SC$\underline{H_2}$Ph), 4.65 (br, 1H, -C$\underline{H}$), 5.1 (s, 2H, -OC$\underline{H_2}$Ph), 5.35 - 5.7 (br, 1H, -N$\underline{H}$), 7.25, 7.35 (s, s, 10H, aromatic protons).

The series of transformations pertaining to the conversion of proline to BzProOMe (11), BzPro-PheOMe (25) and BzPro-Asp(β -OMe)OMe (30) are shown in CHART C.XIII.

BzProOMe (11) :

mp. 89°C

ir : ν_{max}(KBr) cm^{-1} : 1735 (ester), 1615, 1570, 1410.

nmr : $\delta(CDCl_3)$: 1.5 - 2.6 (m, 4H, -CHC$\underline{H_2}$C$\underline{H_2}$), 3.65 (m, 5H, -COOC$\underline{H_3}$, -NC$\underline{H_2}$), 4.45 - 4.85 (br, 1H, -C$\underline{H}$), 6.9 - 8.0 (m, 5H, aromatic).

BzPro-PheOMe (25) :

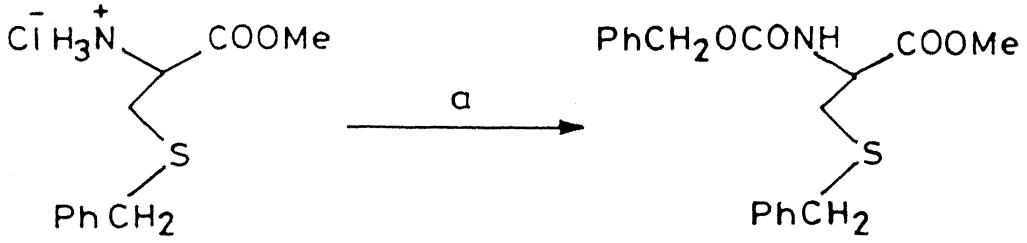
mp. 139 - 142°C

ir : ν_{max}(KBr) cm^{-1} : 3300 (-NH), 1740 (ester), 1670, 1605, 1555 (amide).

nmr : $\delta(CDCl_3)$: 1.55 - 2.45 (br, 4H, -CH(C$\underline{H_2}$)$_2$), 2.95 - 3.25 (m, 2H, -CHC$\underline{H_2}$Ph), 3.25 - 3.6 (m, 2H, -NC$\underline{H_2}$), 3.65 (s, 3H, -COOC$\underline{H_3}$), 4.6 - 5.05 (br, 2H, 2 x -C$\underline{H}$), 7.1 - 8.05 (m, 11H, -N$\underline{H}$, aromatic).

ms : m/z : 380 (M^+), 381 (M^++1)

CHART C-XII

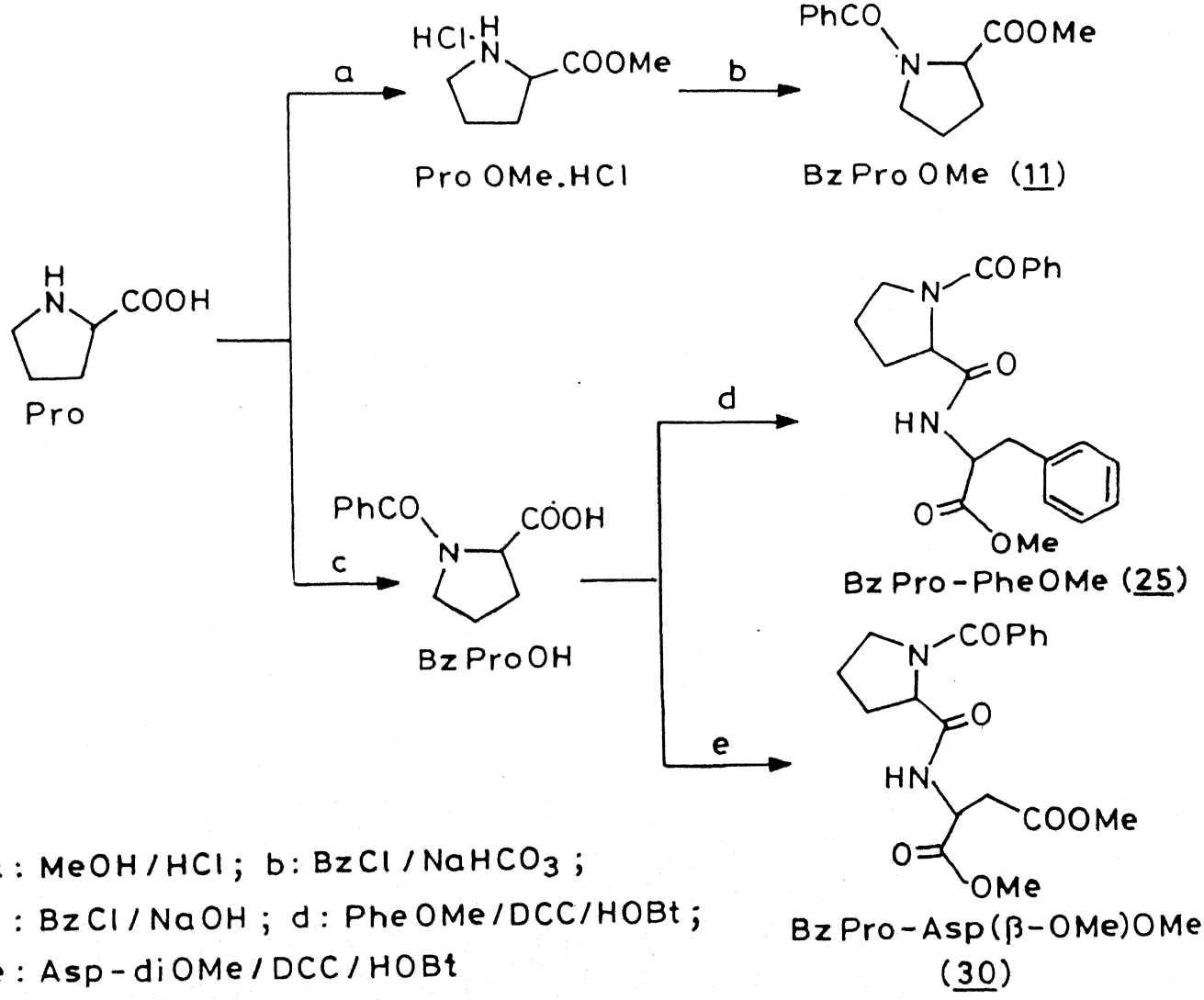


(S-Bzl) Cys OMe.HCl

Z Cys (S-Bzl) OMe (10)

a : Cbz Cl / NaHCO$_3$

CHART C-XIII



a : MeOH / HCl ; b : BzCl / NaHCO$_3$;

c : BzCl / NaOH ; d : Phe OMe / DCC / HOBt ;

e : Asp-di OMe / DCC / HOBt

BzPro-Asp(β -OMe)OMe (30) :

Gummy solid

ir : ν_{max}(neat) cm^{-1} : 3300 (-NH), 1735 (ester), 1675, 1620, 1520 (amide).

nmr : δ($CDCl_3$) : 1.8 - 2.6 (br, 4H, -CH($C\underline{H}_2)_2$), 2.9 (d, 2H, -CHC$\underline{H}_2$COOCH_3), 3.5 - 3.6 (br, 2H, -$(CH_2)_2C\underline{H}_2$), 3.65 (s, 3H, -COOC$\underline{H}_3$), 3.75 (s, 3H, -COOC$\underline{H}_3$), 4.8 (m, 2H, 2 x -C$\underline{H}$), 7.4 (m, 6H, -N$\underline{H}$, aromatic).

ms : m/z ; 362 (M^+), 363 (M^++1)

BzSerOMe (12) and BzThrOMe (13) were prepared from the corresponding amino acids as described in CHARTS C.XIV and C.XV.

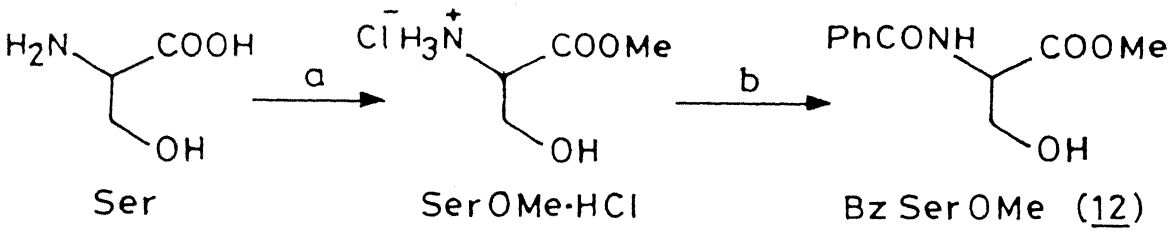
BzSerOMe (12) :

mp. 86°C

ir : ν_{max}(KBr) cm^{-1} : 3430 (-OH), 3300 (-NH), 1740 (ester), 1620, 1530 (amide).

nmr : δ ($CDCl_3$) : 3.15 (br, 1H, -$CH_2O\underline{H}$), 3.7 (s, 3H, -COOC$\underline{H}_3$), 3.95 (d, 2H, -CHC$\underline{H}_2$), 4.8 (m, 1H, -C$\underline{H}$), 7.1 - 8.05 (m, 6H, -N$\underline{H}$, aromatic).

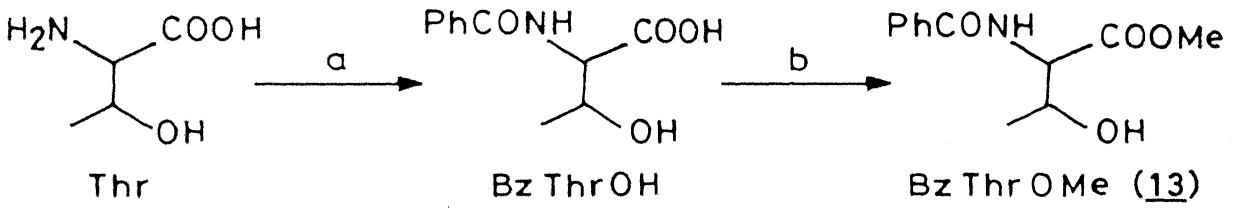
CHART C-XIV



Ser — SerOMe·HCl — BzSerOMe (12)

a: MeOH/HCl ; b: BzCl/NaHCO3

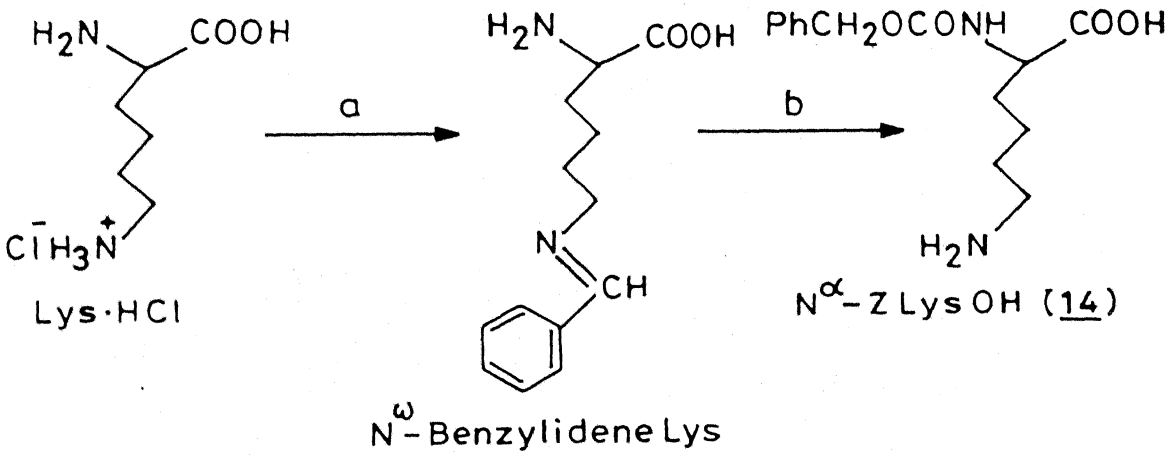
CHART C-XV



Thr — BzThrOH — BzThrOMe (13)

a: BzCl/NaOH ; b: CH_2N_2/MeOH

CHART C-XVI



Lys·HCl — N^{ω}-Benzylidene Lys — N^{α}-Z Lys OH (14)

a: PhCHO/NaOH, b: 1. CbzCl/NaOH, 2. HCl

BzThrOMe (13) :

mp. 91 - 92°C

ir : ν_{max}(KBr) cm^{-1} : 3410 (-OH), 3345 (-NH), 1730 (ester), 1630, 1510 (amide).

Lysine hydrochloride was transformed to N^{α} -Z Lys -OH (14) (CHART C.XVI).

N^{α} -Z Lys -OH (14) :

mp. 232 - 233°C

ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1720 (acid), 1650, 1520 (amide).

The required authentic samples, ZAsp(β -OMe)OMe (17), AcAsp-(β -OMe)OMe (18), BzAsp(β -OMe)OMe (19), as well as that needed for making authentic dipeptides, namely, BzAsp(β -OMe)OH were prepared from aspartic acid as illustrated in CHART C.XVII.

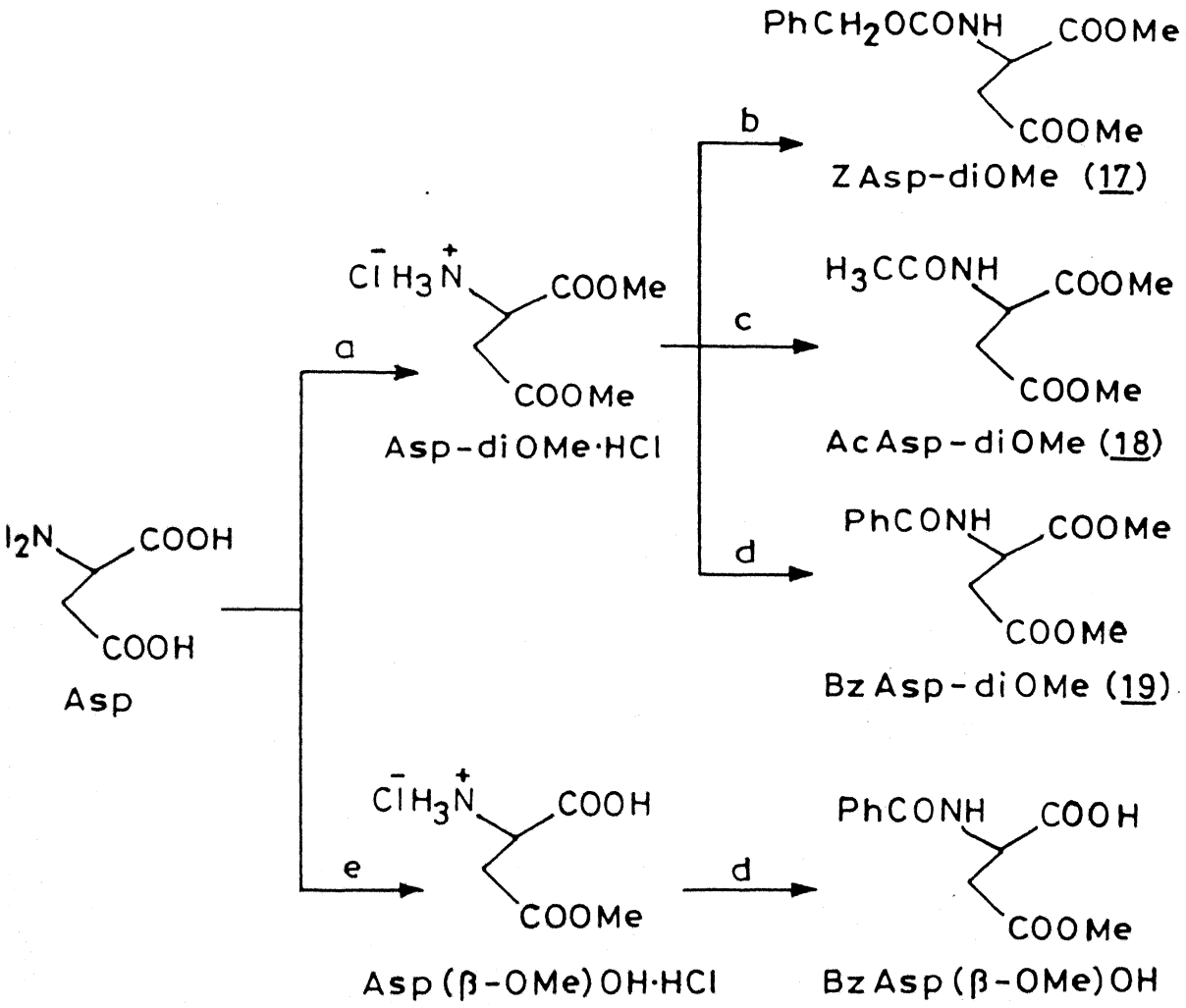
ZAsp(β -OMe)OMe (17) :

Gummy solid

ir : ν_{max}(neat) cm^{-1} : 3330 (-NH), 1710 (ester), 1495, 1430.

nmr : δ ($CDCl_3$) : 2.9 (m, 2H, -CHC$\underline{H_2}$), 3.6 (s, 3H, -COOC$\underline{H_3}$), 3.7 (s, 3H, -COOC$\underline{H_3}$), 4.55 (m, 1H, -C$\underline{H}$), 5.05 (s, 2H, -OC$\underline{H_2}$Ph), 5.65 (m, 1H, -N$\underline{H}$), 7.25 (s, 5H, aromatic).

CHART C-XVII



a : MeOH / HCl ; b : CbzCl / $NaHCO_3$; c : Ac_2O / $NaHCO_3$;

d : BzCl / $NaHCO_3$; e : MeOH / $SOCl_2$

AcAsp(β -OMe)OMe (18) :

mp. 56 - 57°C

nmr : δ ($CDCl_3$) : 2.05 (s, 3H, -COC<u>H</u>$_3$), 2.95 (m, 2H, -CHC<u>H</u>$_2$), 3.7 (s, 3H, -COOC<u>H</u>$_3$), 3.75 (s, 3H, -COOC<u>H</u>$_3$), 4.9 (m, 1H, -C<u>H</u>), 6.45 - 6.95 (br, 1H, -N<u>H</u>).

BzAsp(β -OMe)OMe (19) :

mp. 89°C

ir : ν_{max}(KBr) cm^{-1} : 3280 (-NH), 1725 (ester), 1640, 1520 (amide).

nmr : δ($CDCl_3$) : 3.1 (m, 2H, -CHC<u>H</u>$_2$), 3.7 (s, 3H, -COOC<u>H</u>$_3$), 3.8 (s, 3H, -COOC<u>H</u>$_3$), 5.1 (m, 1H, -C<u>H</u>), 7.2 - 8.0 (m, 6H, -N<u>H</u>, aromatic).

$[\alpha]_D^{25}$: +79.54 (c, 0.44, $CHCl_3$)

BzAsp(β -OMe)OH :

mp. 127°C

ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1750 (ester), 1720 (acid), 1630, 1530.

BzAsp(β -OMe)OH, vide supra, in turn, was transformed to the dipeptides BzAsp(β -OMe)-Asp(β -OMe)OMe (27), BzAsp(β -OMe)-LeuOMe (28) and BzAsp(β -OMe)-PheOMe (29) (CHART C.XVIII).

BzAsp(β -OMe)-Asp(β -OMe)OMe (27) :

mp. 138 - 139°C

ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1750 (ester), 1650, 1550 (amide).

nmr : $\delta(CDCl_3)$: 2.7 - 3.1 (m, 4H, 2 x -CHC<u>H</u>$_2$), 3.6 (s, 3H, -COOC<u>H</u>$_3$), 3.7 (s, 6H, 2 x -COOC<u>H</u>$_3$), 4.6 - 5.25 (br, 2H, 2 x -C<u>H</u>), 6.95 - 8 (m, 7H, -N<u>H</u>, aromatic protons).

BzAsp(β -OMe)-LeuOMe (<u>28</u>) :

Gummy solid

ir : ν_{max}(neat) cm^{-1} : 3260, 1720 (ester), 1620, 1510 (amide).

nmr : $\delta(CDCl_3)$: 0.9 (br, 6H, -CH(C<u>H</u>$_3$)$_2$), 1.6 (br, 2H, -C<u>H</u>$_2$CH(CH$_3$)$_2$), 2.5 (m, 1H, -C<u>H</u>(CH$_3$)$_2$), 2.9 (m, 2H, -C<u>H</u>$_2$COOCH$_3$), 3.7 (s, s, 6H, 2 x -COOC<u>H</u>$_3$), 4.5 (br, 1H, -C<u>H</u>), 5.0(br, 1H, -C<u>H</u>), 6.7 - 8.3 (m, 7H, -N<u>H</u>, aromatic protons).

ms : m/z : 378 (M^+), 379 (M^++1)

BzAsp(β -OMe)-PheOMe (<u>29</u>) :

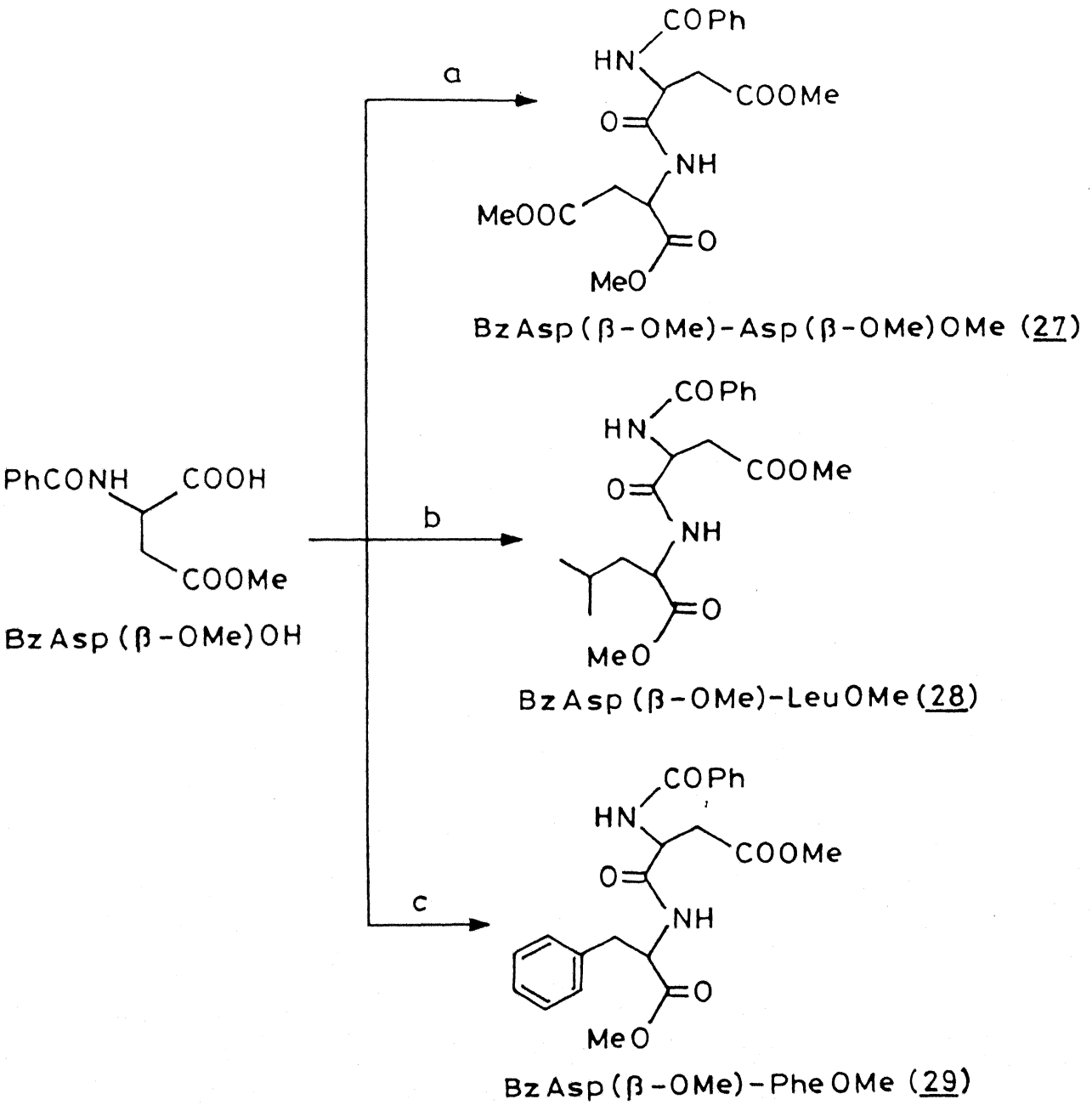
mp. 115 - 117°C

ir : ν_{max}(KBr) cm^{-1} : 3260, 1730 (ester), 1625, 1520 (amide).

nmr : $\delta(CDCl_3)$: 2.7 - 3.25 (m, 4H, 2 x -CHC<u>H</u>$_2$), 3.75 (s, 6H, 2 x -COOC<u>H</u>$_3$), 4.9 (m, 2H, 2 x -C<u>H</u>), 6.6 - 7.9 (m, 12H, -N<u>H</u>, aromatic protons).

ms : m/z : 412 (M^+), 413 (M^++1)

CHART C-XVIII



a: Asp-diOMe/DCC/HOBt; b: LeuOMe/DCC/HOBt;

c: PheOMe/DCC/HOBt

BzGlu(γ -OMe)OMe (20) and BzGlu(γ -OMe)-Asp(β -OMe)OMe (31) were prepared from glutamic acid (CHART C.XIX).

BzGlu(γ -OMe)OMe (20) :

mp. 81°C

ir : ν_{max}(KBr) cm^{-1} : 3260 (-NH), 1730 (ester), 1630, 1525 (amide).

nmr : δ($CDCl_3$) : 1.95 - 2.45 (m, 4H, -CH(CH_2)$_2$), 3.55 (s, 3H, -COOCH_3), 3.65 (s, 3H, -COOCH_3), 4.5 - 4.95 (m, 1H, -CH), 6.95 - 7.95 (m, 6H, -NH, aromatic protons).

ms : m/z : 279 (M^+)

BzGlu(γ -OMe)-Asp(β -OMe)OMe (31) :

Gummy solid

ir : ν_{max}(neat) cm^{-1} : 3310 (-NH), 1730 (ester), 1630, 1525 (amide).

nmr : δ($CDCl_3$) : 2.1 - 2.75 (m, 4H, -CH(CH_2)$_2$), 2.9 (m, 2H, -CHCH_2), 3.65 (s, s, 6H, 2 x -COOCH_3), 3.75 (s, 3H, -COOCH_3), 4.55 - 5.2 (m, 2H, 2 x -CH), 7.1 - 8.1 (m, 7H, -NH, aromatic protons).

ms : m/z : 408 (M^+), 409 (M^++1)

CHART C-XIX

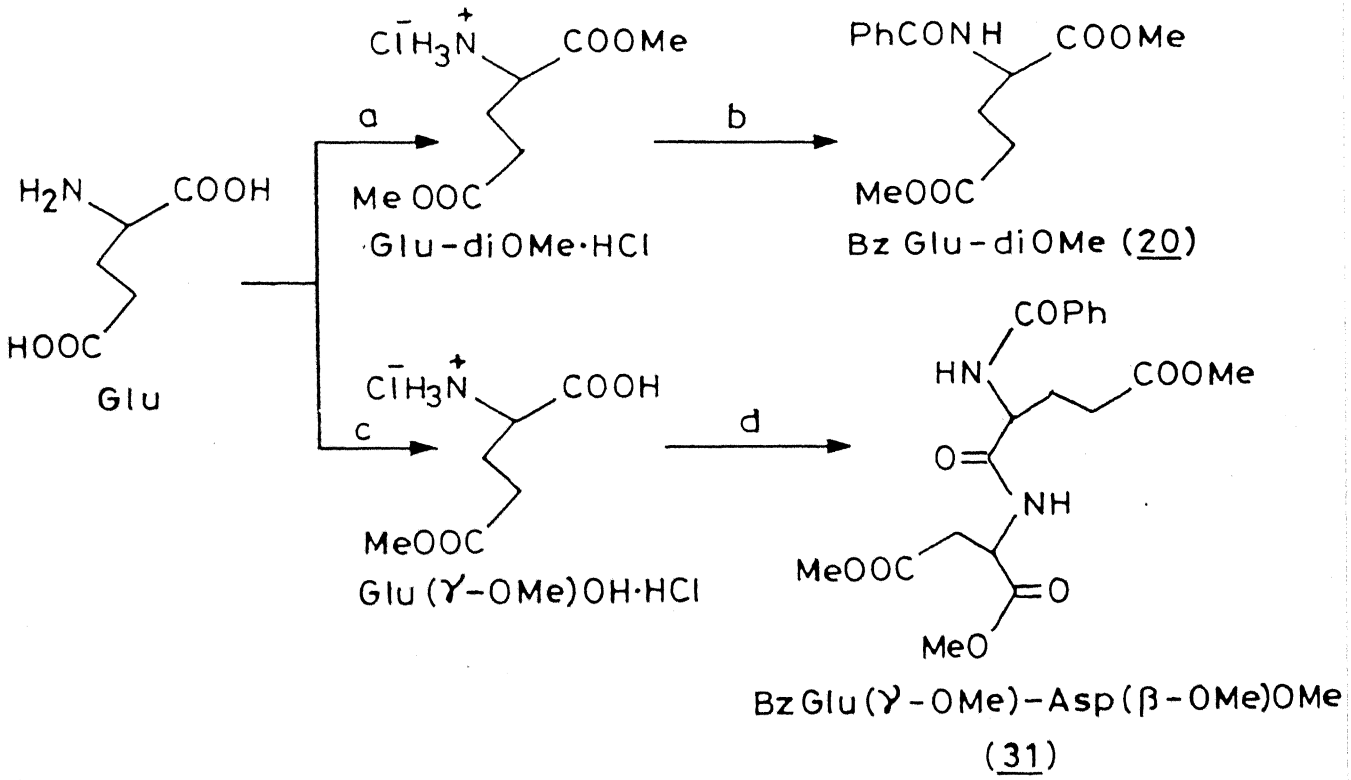
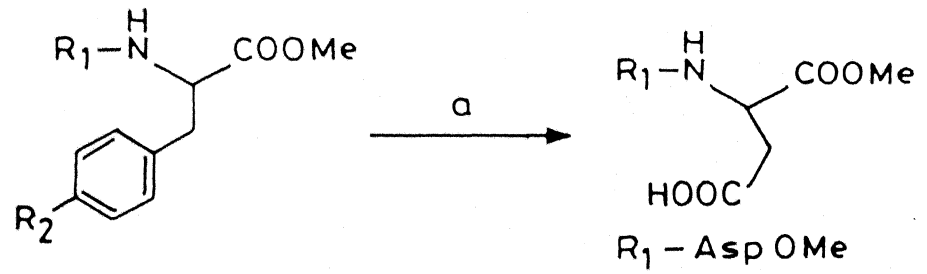


CHART C-XX



1 : R_1= $PhCH_2OCO$, R_2 = H
2 : R_1= H_3CCO, R_2 = H
4 : R_1= PhCO, R_2 = OH

32 : R_1= $PhCH_2OCO$
33 : R_1= H_3CCO
34 : R_1= PhCO

$PhCH_2OCONHCH_2COOMe$ (3) —a→ No reaction

a: $RuCl_3 \cdot 3H_2O$ (2.2 mol %), $NaIO_4$ (18 eq), $MeCN-CCl_4-H_2O$, 60h, rt

B. The oxidation of N,C -protected amino acids :

N-Benzyloxycarbonyl L-phenylalanine methyl ester (1), on treatment with 2.2 mol% of Ru^{VIII} reagent in presence of $NaIO_4$, (18 mmol/mmol of substrate), employing H_2O-MeCN-CCl_4 as the media at rt for 60 h gave ZAspOMe, (32), in 85% yields. The structural assignment for (32) was confirmed via transformation to the dimethyl ester with CH_2N_2 and by comparison with the authentic sample, (17).

ZAsp(β -OH)OMe (32) :

nmr : δ ($CDCl_3$) : 3.0 (m, 2H, -CHC$\underline{H}_2$), 3.7 (s, 3H, -COOC$\underline{H}_3$), 5.1 (s, 2H, -OC$\underline{H}_2$Ph), 4.6 (m, 1H, -C$\underline{H}$), 5.8 (m, 1H, -N$\underline{H}$), 7.2 (s, 5H, aromatic protons).

A noteworthy feature of the (1) → (32) change is the transformation of the side chain aromatic ring in high yields without affecting the phenyl ring of the benzyloxycarbonyl protecting group. The total lack of reactivity of the Z protecting group towards Ru^{VIII} was further established by treatment of ZGlyOMe, (3), under conditions of the (1) → (32) change. The starting material was recovered in nearly quantitative yields and a careful analysis of the reaction mixture demonstrated the absence of any acidic product. This selectivity can be understood on the basis of the marginal deactivation of the aromatic ring present in the Z protecting group, particularly in the context that the unencumbered benzene moiety itself is not a highly susceptible unit. The transformation of BzPheOMe to BzAspOMe (60%) further demonstrates the selectivity in Ru^{VIII} oxidations[51].

Another surprising finding is that even in the case of AcPheOMe, (2),

the oxidation led to AcAspOMe, (33), in 70% yields whose structure was confirmed by esterification with CH_2N_2 and comparison with authentic sample of (18) (CHART C.XX).

N-Benzoyl tyrosine methyl ester, (4), similarly gave BzAspOMe, (34, 75%). When the reaction time was reduced to 12 h, conditions under which (1) is hardly affected, (34) was formed in 68% yields, thus clearly establishing selectivity under competitive environments. The structural assignment was further supported by comparison with an authentic sample of (29). The comparable optical rotation of the oxidation product to that of the authentic sample establishes that chirality is retained throughout.

BzAsp(β -OH)OMe (34) :

mp. 127-128°C

ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1745 (ester), 1710 (acid), 1620, 1520 (amide).

nmr : δ ($CDCl_3$) (400 MHz) : 3.05, 3.2 (dd, dd, 2H, -CHC<u>H</u>$_2$), 3.85 (s, 3H, -COOC<u>H</u>$_3$), 5.05 (m, 1H, -C<u>H</u>), 7.2 - 7.8 (m, 6H, -N<u>H</u>, aromatic protons).

^{13}C-NMR: δ ($CDCl_3$+ DMSO-d_6) (400 MHz) : 171.7, 170.7, 166.2 (3 x -<u>C</u>O), 133.1, 130.8, 127.6, 126.5 (Ph ring carbons), 51.6 (-<u>C</u>H), 48.5 (-<u>C</u>H$_3$), 35.3 (-<u>C</u>H$_2$).

ms : m/z : 251 (M^+)

Apart from the relevance to the objective of the present work, the (1) → (32) change in 85% yields represent, perhaps, the best route to the preparation of N^{α} -protected aspartic acid. This is based on a careful evaluation of the procedures currently available[52].

Another noteworthy feature of the above oxidation is that the reaction is insensitive to the pH of the media in the range 3 to 9. Thus, at pH 3 and pH 9, (34) was isolated in 83% yields in both cases. This compares well with the 84% yield obtained in aqueous media at pH ~ 6 (CHART C.XXI).

Benzoyl tryptophan methyl ester, (5), on oxidation with Ru^{VIII} under conditions of the (1) → (32) change, at 20°C for 60 h gave (34) in 65% yields in addition to N^{α} -benzoyl N^{ω} -formylkynurenine methyl ester (35, 14%) and benzamide (13%). At a higher reaction temperature (~ 35°C), the oxidation resulted in a very good yield of (34), (77%), at the expense of (35) which was absent. ZTrpOMe, (6), on oxidation at rt afforded ZAspOMe (32, 65%) and benzyl carbamate (32%). Parenthetically, oxidative studies on a range of substrates have shown that the Z group is prone to undergo elimination marginally easier compared to the Bz protecting group (CHART C.XXII).

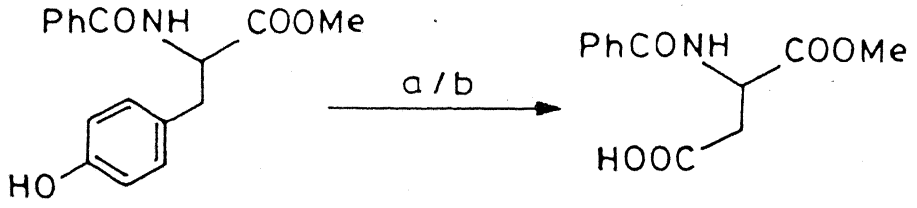
N^{α} -Bz N^{ω} -ForKynOMe (35) :

mp. 94-95°C

ir : ν_{max}(KBr) cm^{-1} : 3310 (-NH), 1750 (ester), 1730 (-CO), 1610, 1655, 1630, 1580, 1510.

nmr : $\delta(CDCl_3)$: 3.85 (s + d, 5H, $-COOC\underline{H}_3$ + $-CHC\underline{H}_2$), 5.15 (m, 1H, -C<u>H</u>), 7.05 - 8.05 (m, 9H, aromatic protons), 8.65 (br, 2H, 2 x -N<u>H</u>), 11.3 (br, 1H, -C<u>H</u>O).

CHART C-XXI

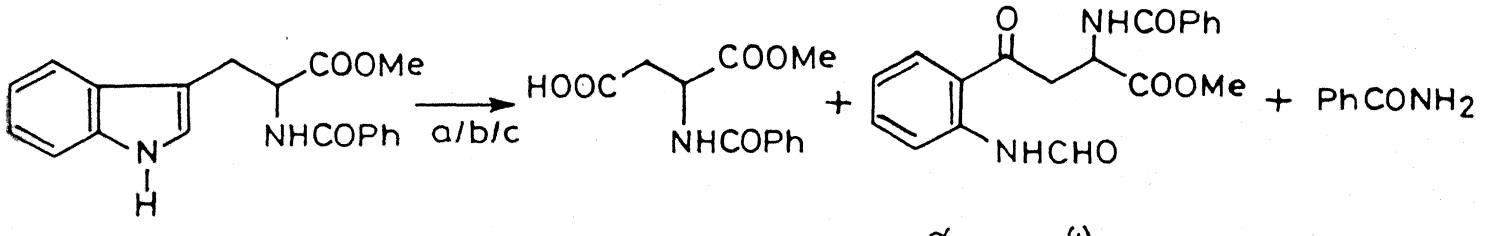


Bz Tyr OMe (4) — Bz Asp OMe (34)

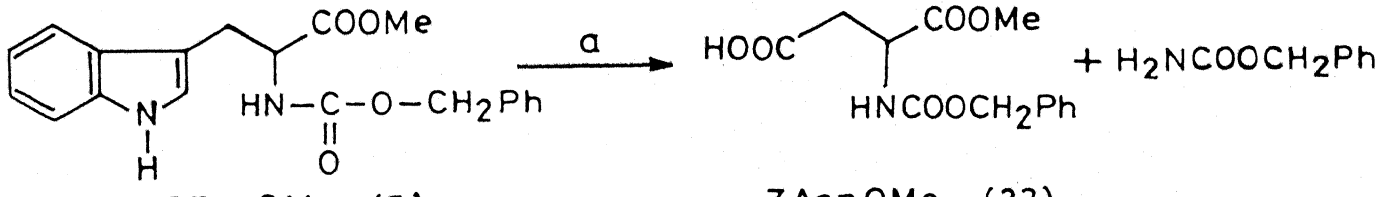
a : $RuCl_3.3H_2O - NaIO_4$, $MeCN - CCl_4$ – Phosphate buffer (pH ~3)

b : $RuCl_3.3H_2O - NaIO_4$, $MeCN - CCl_4$ – Satd. aq. $NaHCO_3$ (pH ~9)

CHART C-XXII



Bz Trp OMe (6) — Bz Asp OMe (34) — N^{α}-Bz, N^{ω}-For Kyn OMe (35)



Z Trp OMe (5) — Z Asp OMe (32)

a: $RuCl_3.3H_2O$ (2.2 mol %), $NaIO_4$ (18 eq), $MeCN - CCl_4 - H_2O$, 60 h, rt

b: 12 h used in 'a' above

c: $NaIO_4$ (2 eq) used in 'a' above ; no (34) was obtained.

^{13}C-NMR: $\delta(CDCl_3)$ (100 MHz) : 200.0, 171.6, 166.1, 161.1 (4 x -CO), 134.5, 134.0, 133.5, 131.3, 130.6, 129.6, 128.1, 127.1, 123.2, 122.7, 115.9 (phenyl ring carbons), 51.9 (CH), 48.6 (CH_3), 40.9 (CH_2).

ms : m/z : 354 (M^+)

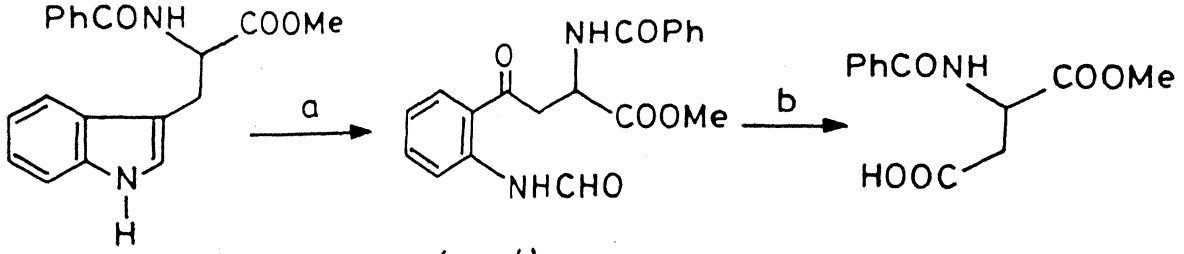
The (6) → (34) change represents an unusual and novel oxidative degradation resulting in the loss of the entire benzenoid moiety. There appears to be no literature precedent wherein such a degradation has been observed[53].

Ancillary experiments have enabled the rationalization of this unusual change on the basis of a sequence involving three distinct types of oxidation by Ru^{VIII}.

The reaction of (6), using normal quantities of oxidizing agents, for a shorter duration of 12 h gave a 62% yield of (34), an enhanced amount of (35) (20%), and no benzamide, thus indicating (35) as a probable intermediate in the overall transformation. This was confirmed via reaction of (6) with only 2 eq. of periodate and 2.2 mol % of Ru-reagent for 60 h at rt, which resulted in the formation of exclusively (35) (47%). The intermediacy of (35) was established by transformation to (34) in 97% yields under conditions of the (1) → (32) change (CHART C.XXIII).

In view of the inertness of the benzenoid moiety in (1), (3) and other such amide substrates, it was considered unlikely that the N-formamido aromatic ring present in (35) could be directly oxidized. On the other hand, the hydrolysis of this formamido grouping, a process that would be facilitated

CHART C-XXIII



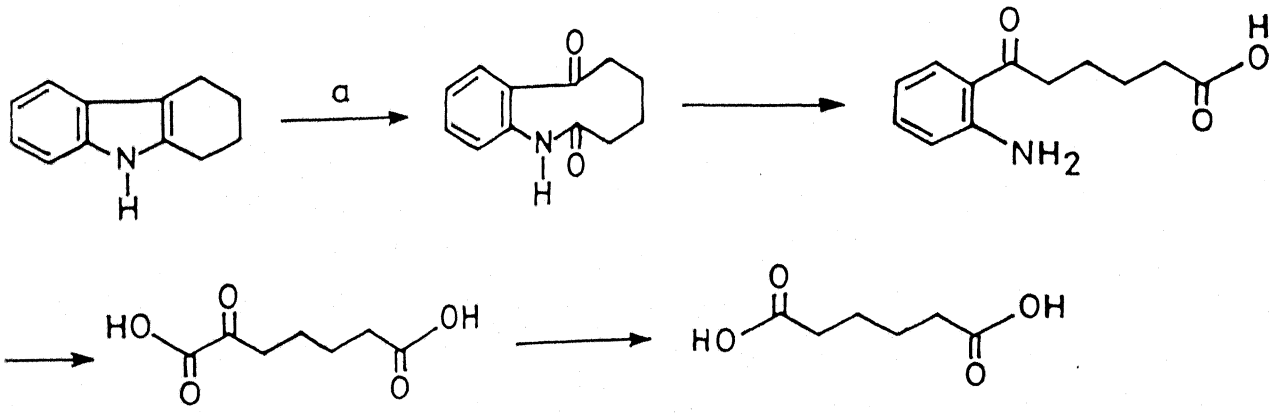
Bz TrpOMe (6)

N^{α}BzN$^{\omega}$-ForKynOMe (35)

BzAsp(β-OH)OMe (34)

a : O_3/AcOH ; b: $RuCl_3 \cdot 3H_2O$ (2.2 mol %), $NaIO_4$ (18 eq), MeCN–CCl_4–H_2O, 60h, rt

CHART C-XXIV



a. $RuCl_3 . 3H_2O$ (2.2 mol %), $NaIO_4$ (18 eq), MeCN–CCl_4–H_2O, 60h, rt

by conjugation with an ene-one type unit[10], would generate an aniline function, a grouping that could be easily degraded to a -COOH unit with Ru^{VIII}.

The noteworthy transformation of tetrahydrocarbazole to adipic acid in 61% yields under conditions of the (6) → (34) change clearly demonstrates that the amide unit arising from the first Ru^{VIII} oxidation is hydrolysed before further degradation. If this were not to be the case, the product would have retained the aryl N-bond. The further oxidation of o-adipoyl aniline, arising from the initial degradation of tetrahydrocarbazole followed by hydrolysis, can be expected to give rise to, by loss of the aromatic moiety, 2-keto pimelic acid. Consequently, the latter would be the appropriate precursor leading to the product, adipic acid (CHART C.XXIV). Thus, the overall process in the (6) → (34) change can be envisaged as taking place via initial oxidation to (35), hydrolysis to N-benzoyl kynurenine methyl ester, further oxidation to N-benzoyl γ -oxoglutamic acid α -methyl ester, and then to (34) by oxidative decarboxylation. In the (6) → (35) change, it so happens that the latent possibilities for the sequential oxidation of the indole ring have found expression leading to its conversion to a carboxyl group (CHART C.XXV).

The oxidative decarboxylation proposed as the last step was tested by attempting to effect a one-step conversion of α -amino acids into lower carboxylic acids by oxidation, since these substrates are usually transformed first into α -keto acids. This was achieved. Valine was transformed into isobutyric acid (66%) under the general oxidation conditions, and phenylalanine to phenylacetic acid (43%) in a shorter reaction (8 h) (CHART C.XXVI)[54].

The experiments cited above pertaining to the novel (6) → (34) change have in addition led to useful reactions of general applicability. Tryptophan residues, either protected, or in a peptide environment, can be transformed

CHART C-XXV

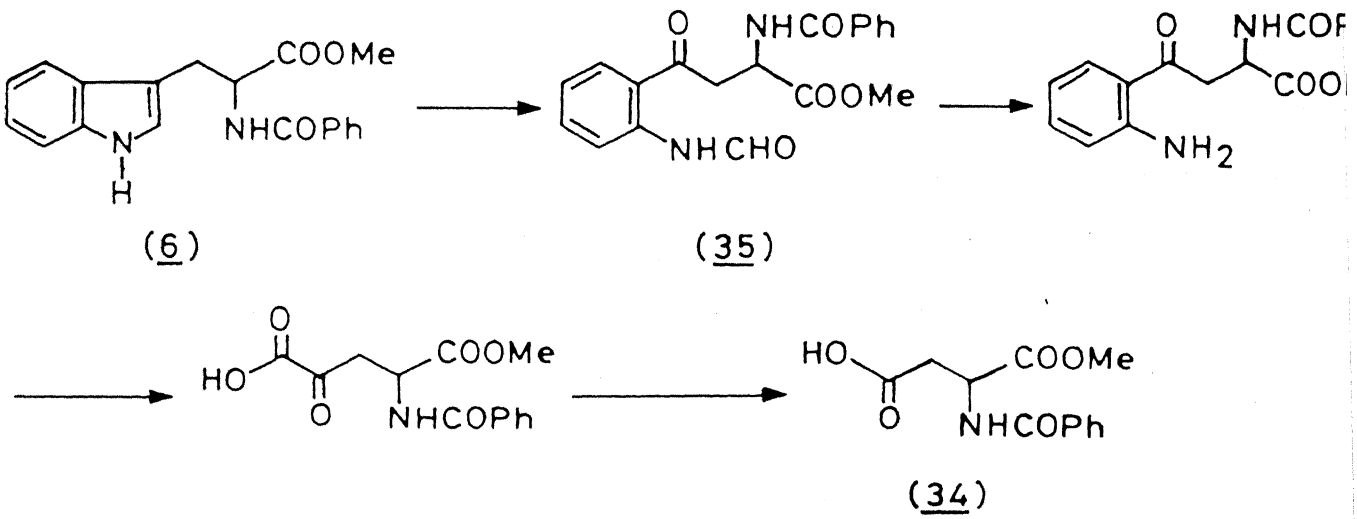
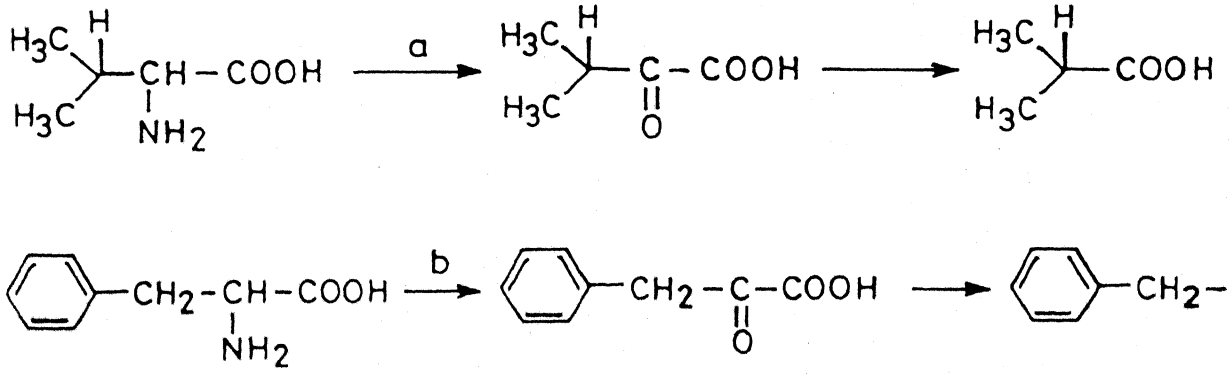


CHART C-XXVI



a. $RuCl_3 \cdot 3H_2O$ (2.2 mol %), $NaIO_4$ (18 eq), 60h, rt.

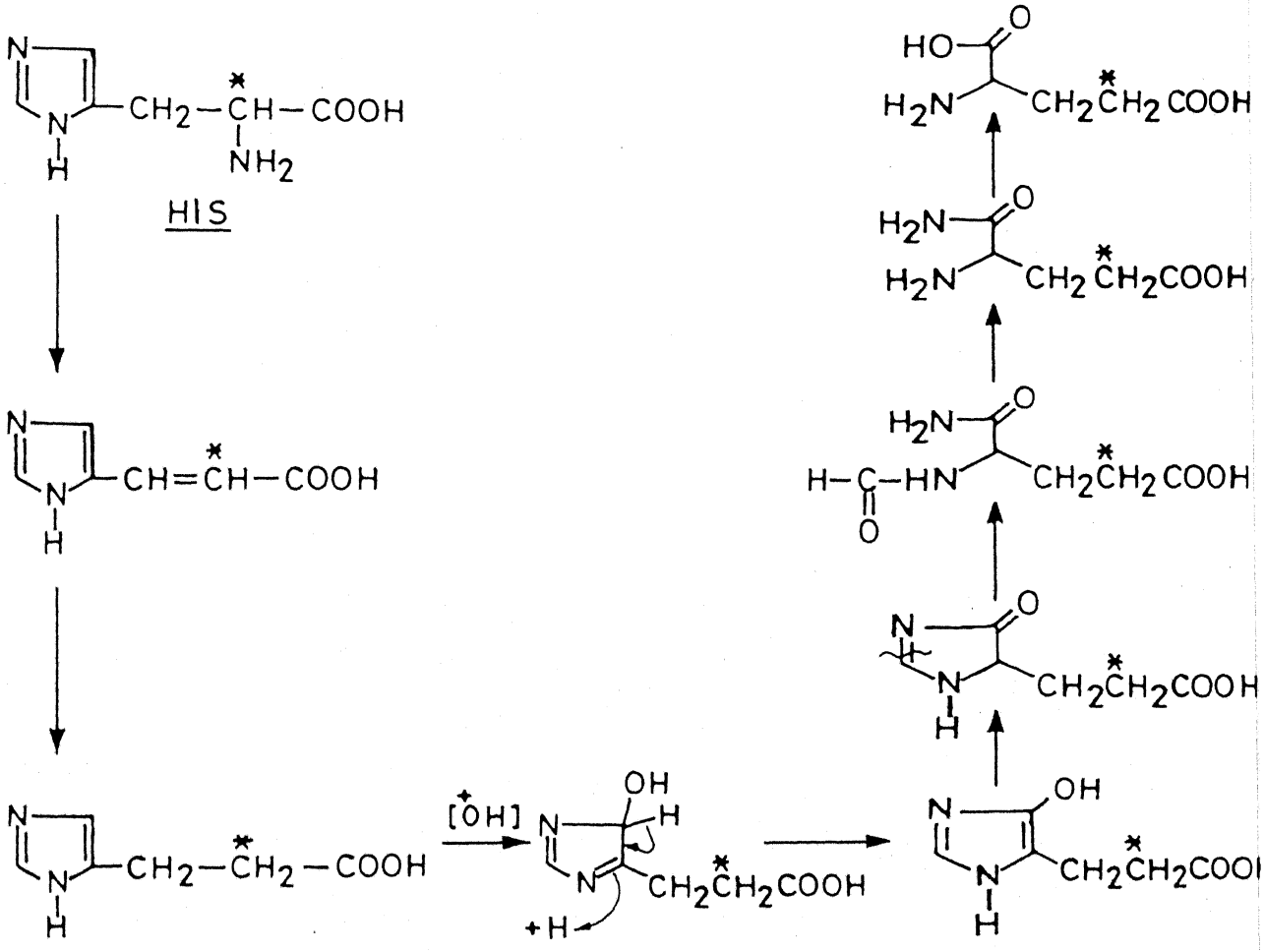
b. $RuCl_3 \cdot 3H_2O$ (2.2 mol %), $NaIO_4$ (18 eq), 8h, rt.

to N-formyl kynurenines. The kynurenines, in turn, produced in this manner, or via the numerous methodologies cited in sub-part B, can be transformed to aspartic acid residues with ease. The ready one-step transformation of α-amino acids to lower carboxylic acids illustrated with phenylalanine and valine can be expected to be of use in preparative, analytical and degradation studies of other α -amino acids or such units present in other substrates. Parenthetically, the degradative studies pertaining to free amino acids are presented under the sub-heading 'F' (vide supra).

Histidine offers a cornucopia of opportunities but, at the same time, poses severe impediments pertaining to site specific oxidative transformations. Four of the five atoms of the imidazole ring of histidine are sites for hyperreactivity involving a range of reagents. Thus, electrophiles, nucleophiles, oxidizing and reducing agents and molecular oxygen readily react with this unit to generate a range of products. At the outset, it was envisaged that the oxidation of histidine would lead to aspartic acid as well as other novel side chain residues. Indeed, the work on the oxidation of N,C -protected histidines carried out previously[51], and in the present work, vide infra, fully confirmed the above expectations.

The oxidative transformations of histidine and related compounds, both in vivo and in vitro environments, have been the object of scrutiny for a number of years (CHARTS B.10 and B.11). Of relevance to the present work is the transformation of histidine to glutamic acid in liver and mitochondria, involving, as a key step, the hydroxylation of the imidazole 4-position (CHART C.XXVII)[55]. Although such an imidazole 4-hydroxylation was encountered in the course of studies with the ozone equivalent 4-^{t}butyliodoxybenzene (CHART B.11), RuO_4 invariably oxidized the 2-location of the histidine imidazole

CHART C-XXVII



as well as the model compound tetrahydrobenzimidazole, leading to a range of closely related structures.

The reaction of N-benzyloxycarbonyl histidine methyl ester (7) with Ru^{VIII} species under conditions described previously, gave, ZAspOMe (32) in 25% yields and the novel amino acid N-benzyloxycarbonyl aspartoyl urea α -methyl ester (36, 22%). The structural assignment for (36) is supported by spectral and analytical data.

ZAsn(β $-CONH_2$)OMe (36) :

mp. 168-169°C

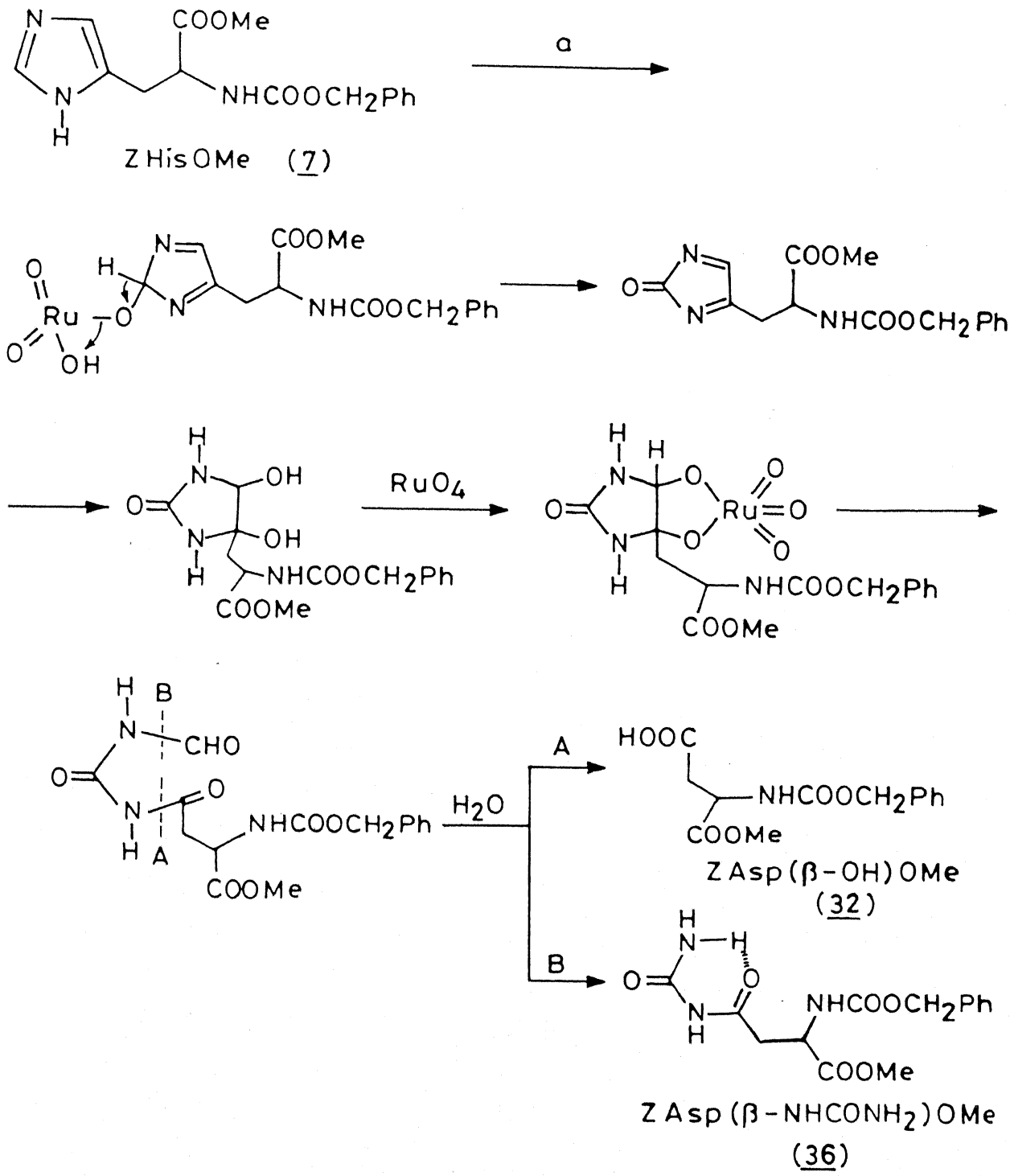
ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1740 (ester), 1690, 1660, 1530 (amide).

nmr : δ ($CDCl_3$ + DMSO $-d_6$) : 2.8 (d, 2H, $-CHC\underline{H}_2$), 3.65 (s, 3H, $-COOC\underline{H}_3$), 4.3 - 4.7 (m, 1H, $-C\underline{H}$), 5.05 (s, 2H, $-OC\underline{H}_2Ph$), 6.7-7.1 (br, 2H, $-CO-N\underline{H}_2$), 7.3 (s, 5H, aromatic protons), 7.8-8.1 (m, 1H, $-CHN\underline{H}$), 10.1 (s, 1H, $-CONHCONH_2$).

ms : m/z : 323 (M^+), 324 (M^++1)

The (7) → (32) + (36) transformation had to be reconciled with previous studies[51] on the oxidation of N -benzoyl histidine methyl ester, resulting in the formation of, in addition to (34) (34%), N^{α} -benzoyl N^{ω} -formyl asparagine methyl ester (26%) and N -benzoyl β -oxoglutamine methyl ester (11%). Regardless of the finer details of the reaction mechanism, the (7) → (36) change must

CHART C - XXVIII



a : $RuCl_3.3H_2O$ (2.2 mol %), $NaIO_4$ (18 eq), $MeCN-CCl_4-H_2O$, 60h, rt

involve imidazole 2-oxidation, whilst that pertaining to BzHisOMe should necessarily invoke acceptance of RuO_4 at the imidazole 4-location. Superimposed on this is the fact that tetrahydrobenzimidazole, under conditions of the (7) → (32) + (36) change, yielded, 4,5 -dihydroxy hexahydro benzimidazole 2-one which should entail oxidation at the 2-location[56]. In 4(5) - substituted imidazoles such as present in (7) and BzHisOMe, the nucleophilic sites available at 2- and 5(4) - locations are the logical sites for oxidation. The observed experimental fact that in the case of tetrahydrobenzimidazole where the 4(5) positions are substituted the oxidation takes place exclusively at the 2-location not only supports the above conclusions, but also tends to suggest that steric factors arising from 4(5) - substitution, can lead to preferential 2-oxidation. Consequently, the divergence in results shown with respect to (7) and BzHisOMe can be rationalized on the basis of the bulkier benzyloxycarbonyl protecting group present in (7). The formation of (32) and (36), on the basis of a common 2-oxo intermediate, is rationalized in CHART C.XXVIII.

The preferential acceptance of elements of RuO_4 at the 2-location of (7), (vide supra), should lead to the highly electrophilic 2,4 -bisaza cyclopentadienone common intermediate, similar to that envisaged in the tetrahydrobenzimidazole oxidation. This readily accepts elements of water and the resulting vic-diol undergoes RuO_4-mediated cleavage. The resulting product, via hydrolysis by pathways (A) and (B), would lead to, respectively, (32) and (36) (CHART C.XXVIII).

The oxidation of methionine side chains is an important biological process and, in many cases, could alter the activity profile of an enzyme. This aspect is illustrated with the enzyme α_1-antitrypsin, the absence of which could result in fatal adult respiratory syndrome. α_1-Antitrypsin, a glycosated serum protein containing 391 amino acid residues possesses 8 methio-

nine side chains, the oxidation of even one of which, particularly the one at 358 location, results in dramatic reduction of enzyme activity. Recently, site directed mutagenesis at this location involving the Met → Val substitution produced an oxidation resistant derivative, an observation that demonstrates the potential of engineered modifications of protein molecules to improve their physiological function[57].

In the event, the oxidation of N-benzoyl methionine methyl ester (8) with Ru^{VIII} yielded, most surprisingly, a 65% yield of BzMet (SO_2)OH (38), and a mere 7% of the corresponding ester (37). Several blank experiments clearly demonstrated that a prior S-oxidation is necessary for the ester hydrolysis. Earlier work using 4-^{t}butyl iodoxybenzene also resulted in ester hydrolysis, although in modest yields (CHART B.13). The unusual transformation of (8) to (38) is rationalized on the basis of an intramolecular cyclization of the initially formed sulfoxide, followed by hydrolysis of the resulting activated ester and further oxidation (CHART C.XXIX).

BzMet(SO_2)OMe (37) :

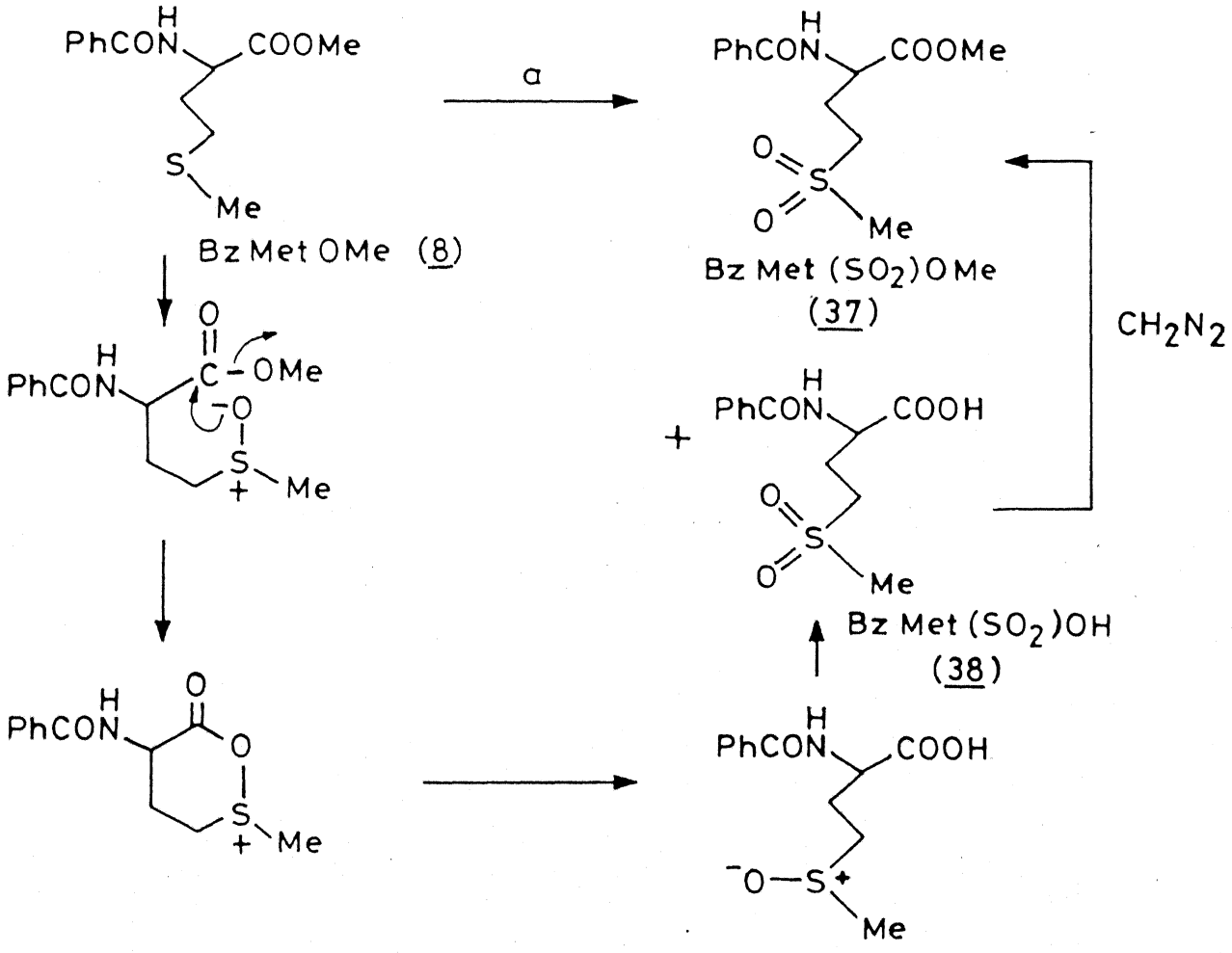
mp. 118 - 119°C

ir : ν_{max}(KBr) cm^{-1} : 3350 (-NH), 1760 (-CO), 1660, 1550 (amide), 1310, 1255, 1145 ($-SO_2$).

nmr : δ ($CDCl_3$) : 2.9 (s, 3H, $-SO_2\underline{CH_3}$), 3.0 - 3.4 (m, 4H, $-CH(\underline{CH_2})_2$), 3.75 (s, 3H, $-COO\underline{CH_3}$), 4.9 (q, 1H, -CH), 7.1 - 7.9 (m, 6H, -NH, aromatic protons).

ms : m/z : 299 (M^+)

CHART C-XXIX



a : $RuCl_3.3H_2O$ (2.2 mol %), $NaIO_4$ (18 eq), MeCN - CCl_4 - H_2O, 60 h, r t

The further transformations of the reactive cyclic intermediate arising from intramolecular cyclization of the initially formed sulfoxide is governed by subtle factors. This aspect was uncovered during parallel studies with N-benzyloxycarbonyl methionine methyl ester, (9), which gave, under conditions described previously, in addition to the expected sulfone (39), (40%), N-methyloxycarbonyl methionine sulfone methyl ester (40, 18%). This rather intriguing change, amounting to the replacement of the benzyloxycarbonyl protecting group present in the starting material by a methoxycarbonyl unit present in product (40), can be best rationalized on the basis of an intramolecular opening of the reactive cyclic intermediate referred to above by the relatively more nucleophilic carbamate protecting group. The resulting azlactone, on hydrolysis, further oxidation, bicarbonate opening and CH_2N_2 esterification, would yield the observed product (40) (CHART C.XXX).

ZMet(SO_2)OMe (39) :

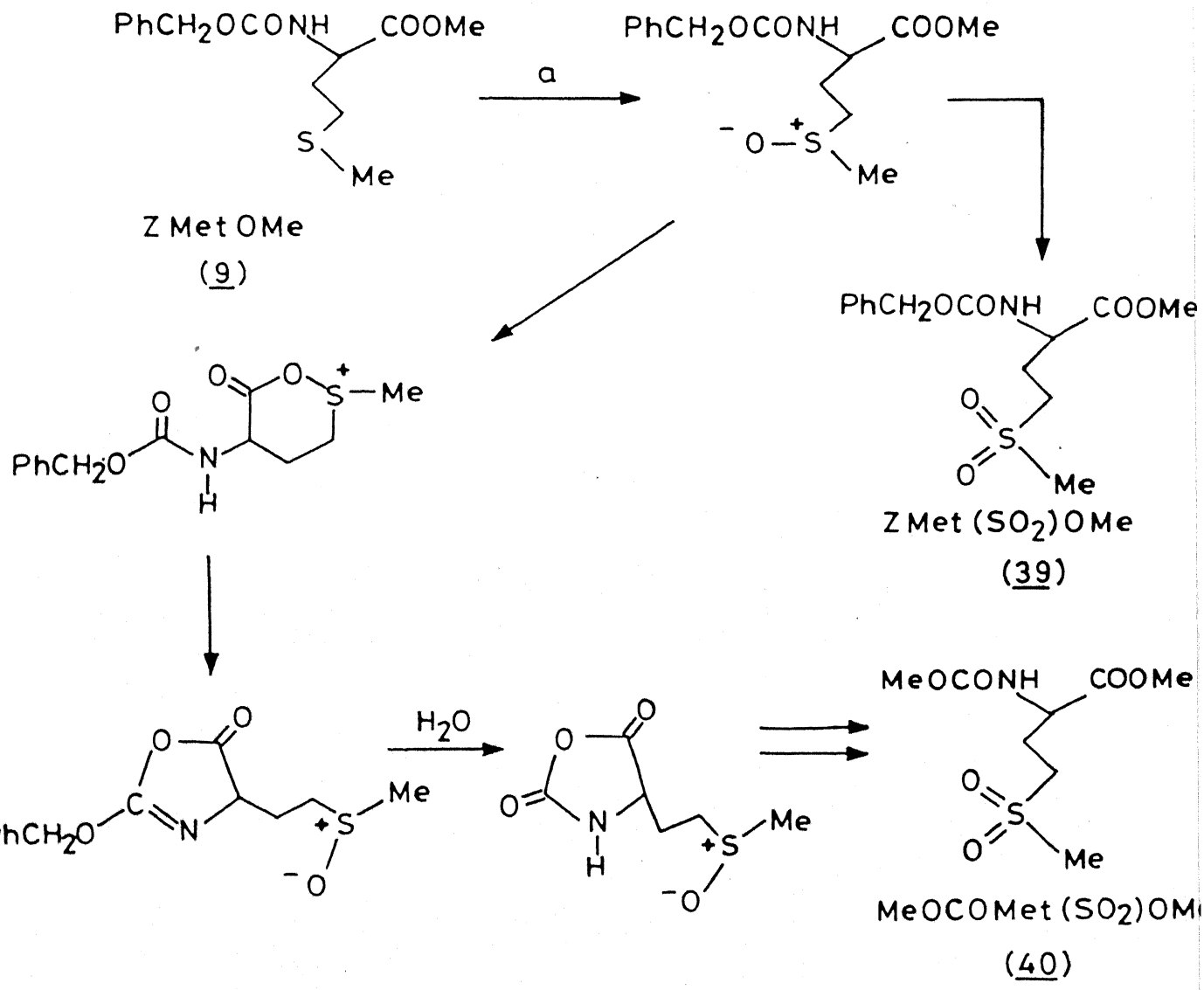
mp. 89°C

ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1730 (ester), 1690, 1530, 1320, 1270, 1125 (SO_2).

nmr : $\delta(CDCl_3)$: 2.0 - 2.55 (m, 2H, -CHC$\underline{H_2}$), 2.8 (s, 3H, -$SO_2C\underline{H_3}$), 2.9 -3.3 (m, 2H, - $C\underline{H_2}SO_2CH_3$), 3.7 (s, 3H, -COOC$\underline{H_3}$), 4.1 - 4.65 (m, 1H, -C$\underline{H}$), 5.05 (s, 2H, -OC$\underline{H_2}$Ph), 5.35 - 5.9 (br, 1H, -N$\underline{H}$), 7.25 (s, 5H, aromatic protons).

ms : m/z : 329 (M^+), 330 (M^++1)

CHART C-XXX



a: $RuCl_3 . 3H_2O$ (2.2 mol %), $NaIO_4$ (18 eq), $MeCN-CCl_4-H_2O$
60 h, rt.

MeOCO-Met(SO_2)OMe (40) :

Thick syrup

ir : ν_{max}(neat) cm^{-1} : 3310 (-NH), 1725, 1520, 1440.

nmr : δ($CDCl_3$ + DMSO -d_6) : 2.05 - 2.65 (br, 2H, -CHC$\underline{H_2}$), 2.9 (s, 3H, -$SO_2C\underline{H_3}$), 3.15 (t, 2H, -$C\underline{H_2}SO_2CH_3$), 3.65 (s, 6H, 2 x -$CO_2C\underline{H_3}$), 4.7 - 5.0 (br, 1H, -C$\underline{H}$).

ms : m/z : 253 (M^+)

The reaction of N-benzyloxycarbonyl S-benzyl cysteine methyl ester, (10), with Ru^{VIII} gave the sulfone ester (41) in 30% yields. The corresponding ZCys (SO_2-Bzl)OH could not be detected, possibly because of difficulties associated with the realization of the transition state leading to intramolecular cyclization with concomittant displacement of the methoxyl group (CHART C.XXXI).

ZCys (SO_2-Bzl)OMe (41) :

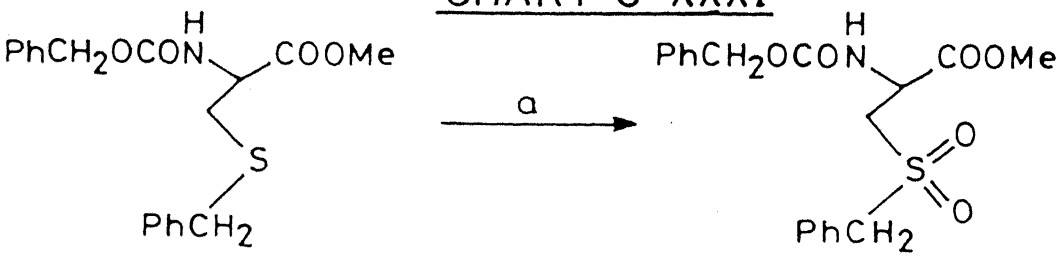
mp. 174 - 175°C

ir : ν_{max}(KBr) cm^{-1} : 3315 (-NH), 1730 (ester), 1685, 1520, 1300, 1250, 1130 (SO_2).

nmr : δ ($CDCl_3$) : 3.5 (d, 2H, -CHC$\underline{H_2}$), 3.75 (s, 3H, -COOC$\underline{H_3}$), 4.2 (s, 2H, -$SO_2C\underline{H_2}$Ph), 4.65 - 4.85 (br, 1H, -C$\underline{H}$), 5.1 (s, 2H, -OC$\underline{H_2}$Ph), 5.85 (br, 1H, -N$\underline{H}$), 7.2 - 7.4 (m, 10H, aromatic protons).

ms : m/z : 391 (M^+)

CHART C-XXXI

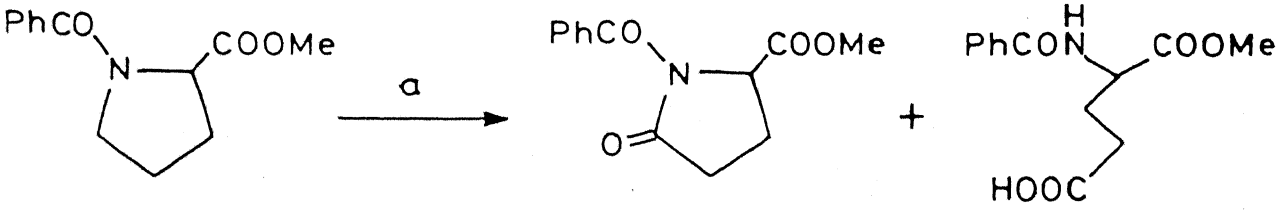


Z Cys (S-Bzl) OMe
(10)

Z Cys (SO_2-Bzl) OMe
(41)

a: $RuCl_3.3H_2O$ (2.2 mol %), $NaIO_4$ (18 eq), $MeCN-CCl_4-H_2O$, 60 h, rt

CHART C-XXXII



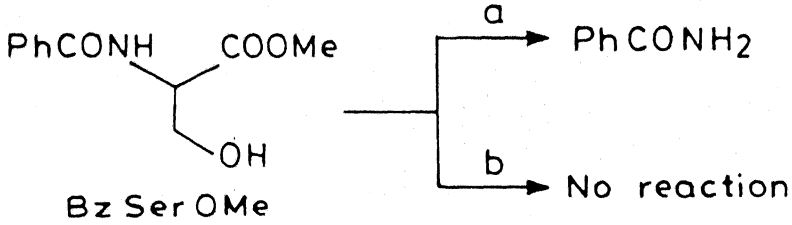
Bz Pro OMe
(11)

Bz-pyroGlu OMe
(42)

Bz Glu (γ-OH) OMe
(43)

a: $RuCl_3.3H_2O$ (2.2 mol %), $NaIO_4$ (18 eq), $MeCN-CCl_4-H_2O$, 60 h, rt

CHART C-XXXIII



Bz Ser OMe
(12)

a: $RuCl_3.3H_2O-NaIO_4$, $MeCN-CCl_4-H_2O$, 60 h, rt

b: $MeCN-CCl_4$-Phosphate buffer (pH~3) used in 'a' above

vage having potential for protein rupture at these sites.

N-benzoyl serine methyl ester (12) exhibited a dual reaction profile with Ru^{VIII}. Whilst no reaction was observed under usual conditions of oxidation in pH 3 phosphate buffer, in water, the oxidation led to the formation of benzamide (58%) (CHART C.XXXIII).

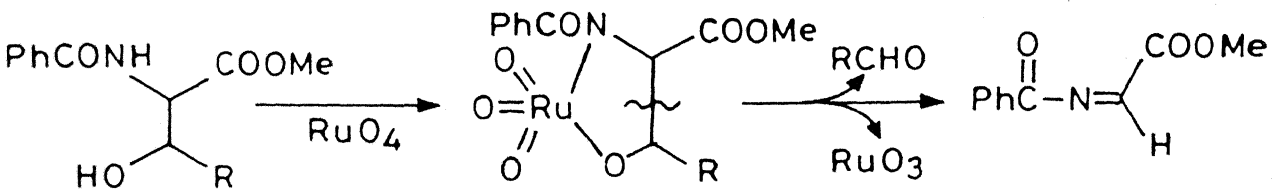
In sharp contrast, the oxidation of N-benzoyl threonine methyl ester (13) with Ru^{VIII} species in pH 3 phosphate buffer gave a 84% yield of benzamide. A similar transformation took place in aqueous media affording benzamide in lower yields (25%) (CHART C.XXXIV).

The formation of benzamide in the reactions of (12) and (13) can be rationalized on the basis of a Ru-complex incorporating the vicinal amino-alcohol unit followed by fragmentation and hydrolysis (CHART XXXV). A logical extension of the above rationalization indicates that this oxidative methodology has potential as a possible method of N-terminal rupture of serine and threonine peptides. This degradation would be of significance since both serine and threonine are common side chain residues encountered in peptides and, therefore, rupture at these sites would provide a valuable method for the preparation of smaller peptide fragments which could be judiciously used in the synthesis of a variety of proteins by restructuring[49].

The possible oxidative transformations of N,C -protected asparagines and glutamines were of interest because in the event the reaction took place, it would amount to a differentiation between the primary and secondary amides that are present in the substrates.

The reaction of N-benzyloxycarbonyl asparagine methyl ester (15) with Ru^{VIII} under usual conditions resulted in a complex mixture from which

CHART C-XXXIV



(12): R = H

(13): R = Me

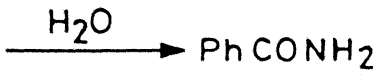
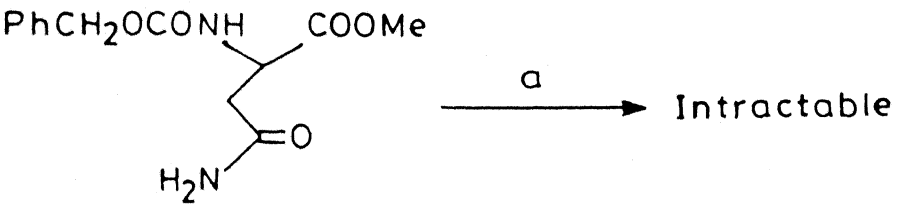
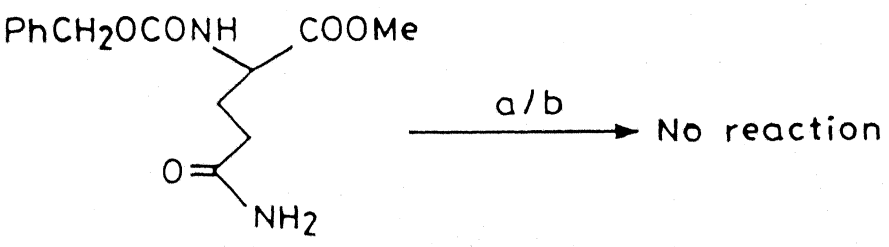


CHART C-XXXV



Z Asn OMe
(15)



Z Gln OMe
(16)

a: $RuCl_3.3H_2O - NaIO_4$, $MeCN - CCl_4 - H_2O$, 60h, rt

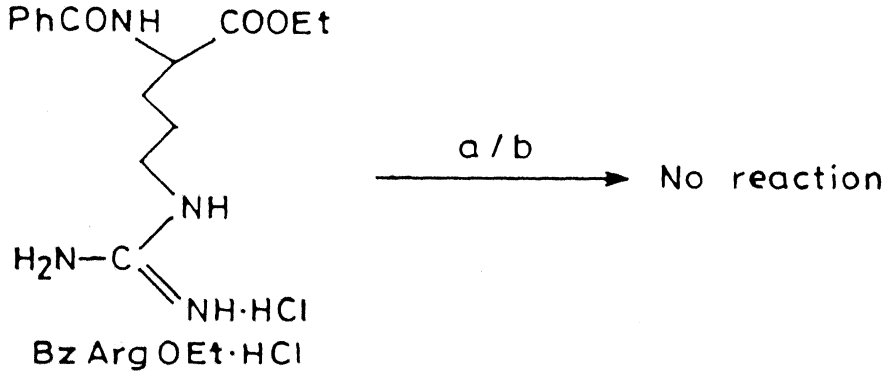
b: $MeCN - CCl_4$ – Phosphate buffer (pH~3) used in 'a', 18h, rt

no pure products could be isolated. In sharp contrast, similar studies with N-benzyloxycarbonyl glutamine methyl ester (16), either in aqueous media or in pH 3 phosphate buffer for 18 h, resulted in the recovery of the starting material. These experiments would give confidence that glutamine side chains present in peptides would not be oxidized (CHART C.XXXV).

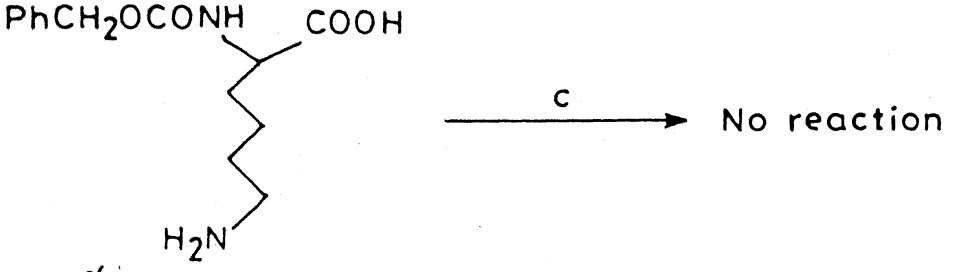
As stated previously, one of the objectives of the present study pertaining to the oxidation of N,C - protected coded amino acids with Ru^{VIII} was to delineate conditions under which a susceptible group can be made inert by control over the reaction conditions. Arginine and lysine offered excellent examples to illustrate this point. The present studies, as well as those available in the literature, would indicate that in a protonated state, the amino group is not usually amenable to Ru^{VIII} oxidation in sharp contrast to the free NH_2 system that undergoes oxidation with extraordinary ease. As anticipated, the reaction of BzArgOEt.HCl, either at pH 6 under the normal conditions of oxidation for prolonged periods (100 h), as well as in phosphate buffer at pH 3 for 60 h, resulted in no reaction. This was not surprising, since, under the conditions under which the reaction was carried out, the arginine moiety would be protonated throughout. In a similar manner, the reaction of N^{α} -benzyloxycarbonyl lysine (14), in phosphate buffer (pH ~ 3) for 18 h,led to recovery of the starting material (CHART C.XXXVI).

The oxidative transformations on N,C - protected amino acids thus far carried out are presented in TABLE C-I. The studies referred to above clearly suggest possibilities for preferential oxidation in a competitive environment, either on the basis of higher susceptibility of the side chain, or on the basis of pH control. These expectations could be realized by studies with a range of dipeptides.

CHART C-XXXVI



Bz Arg OEt·HCl



N^{α}-Z Lys OH

(14)

a : $RuCl_3 \cdot 3H_2O - NaIO_4$, $MeCN - CCl_4 - H_2O$, 100 h, rt

b : $MeCN - CCl_4$ - Phosphate buffer (pH~3) used in 'a', 60 h, rt

c : Same as 'b', 18 h, rt

TABLE C-I

S. No	SUBSTRATE	REACTION TIME (h)	PRODUCT/S	YIELD (%)
1	Z Phe OMe (1)[a]	60	Z Asp(β-OH)OMe (32)	85
2	Z Gly OMe (3)[a]	60	No change	-
3	Ac Phe OMe (2)[a]	60	Ac Asp(β-OH)OMe (33)	70
4	Bz Tyr OMe (4)[a]	60	Bz Asp(β-OH)OMe (34)	84
5	Bz Tyr OMe (4)[a]	12	Bz Asp(β-OH)OMe (34)	69
6	Bz Tyr OMe (4)[b/c]	18	Bz Asp(β-OH)OMe (34)	83
7	Bz Trp OMe (5)[a]	60	Bz Asp(β-OH)OMe (34)	65
			N^{α}-Bz N^{ω}-For-Kyn OMe (35)	14
			$PhCONH_2$	13
8	Bz Trp OMe (5)[a]	12	Bz Asp(β-OH)OMe (34)	62
			N^{α}-Bz N^{ω}-For-Kyn OMe (35)	20
9	Bz Trp OMe (5)[d]	60	N^{α}-Bz N^{ω}-For-Kyn OMe (35)	47
10	Z Trp OMe (6)[a]	60	Z Asp(β-OH)OMe (32)	65
			$PhCH_2$-O-$CONH_2$	32
11	Z His OMe (7)[a]	60	Z Asp(β-OH)OMe (32)	25
			Z Asn($CONH_2$)OMe (36)	22
12	Bz Met OMe (8)[a]	60	Bz Met(SO_2)OMe (37)	7
			Bz Met(SO_2)OH (38)	65

contd. TABLE C-I

S. No	SUBSTRATE	REACTION TIME (h)	PRODUCT /S	YIELD (%)
13	Z Met OMe (9)[a]	60	Z Met (SO_2) OMe (39) MeOCOMet (SO_2) OMe (40)	40 18
14	Z Cys (S-Bzl) OMe (10)[a]	60	Z Cys (SO_2 - Bzl) OMe (41)	30
15	Bz Pro OMe (11)[a]	60	Bz - Pyro Glu - OMe (42) Bz Glu (γ -OH) OMe (43)	40 17
16	Bz Ser OMe (12)[a]	60	$PhCONH_2$	58
17	Bz Ser OMe (12)[b]	72	No change	-
18	Bz Thr OMe (13)[a]	60	$PhCONH_2$	25
19	Bz Thr OMe (13)[b]	18	$PhCONH_2$	84
20	Z Asn OMe (15)[a]	60	Intractable	-
21	Z Gln OMe (16)[a]	60	No change	-
22	Z Gln OMe (16)[b]	18	No change	-
23	Bz Arg OEt · HCl[a]	100	No change	-
24	Bz Arg OEt · HCl[b]	60	No change	-
25	N^{α} - Z Lys OH (14)[b]	18	No change	-

"a": $MeCN : CCl_4 : H_2O$ :: 4 : 4 : 8 ml, $NaIO_4$ (18 mmol) / mmol of substrate ; $RuCl_3 \cdot 3H_2O$ (2.2 mol %) at rt.

"b": Phosphate buffer (pH ~ 3) used insted of H_2O in "a"

"c": Satd. aq. $NaHCO_3$ (pH ~ 9) used insted of H_2O in "a"

"d": $NaIO_4$ (2 mmol) used in "a" above.

C. The oxidation of dipeptides and a tetrapeptide under competitive and non-competitive circumstances :

In a peptide environment, alongwith the transplantation of experience gained from studies on N,C - protected amino acids, several points need clarification. An important aspect would be that since most of the side chain studies are transformations to aspartic acid, the overall change would amount to the complete profile alteration of the side chain polarity and, consequently, in other properties. For example, in dipeptides carrying additional oxidizable side chain residues, the first oxidation could create experimental problems. Fortunately, this appears to be not true since earlier work[51] had shown that BzPhe-PheOMe and BzPhe-Phe-PheOMe could be smoothly transformed to BzAsp-AspOMe and BzAsp-Asp-AspOMe. Since the above represent systems that could offer maximum complexity, it was concluded that Ru^{VIII} mediated oxidations could be confidently used in peptides having two or more susceptible side chains.

A mixture of rational analysis and intuitive considerations pertaining to the examples cited in TABLE C-I would show that a clear reactivity pattern exists. Whilst the most susceptible residue would certainly be methionine, the others could perhaps be arranged in the following order of decreasing reactivity under normal oxidation conditions : tryptophan, histidine, tyrosine, phenylalanine, proline. In a similar manner, in pH 3 phosphate buffer, the order could be anticipated to be, in a decreasing order, tryptophan, tyrosine, phenylalanine, histidine, proline, lysine, arginine. These expectations have been fully realized on studies with di and higher peptides.

The oxidation of BocPhe-PheOMe (21) was studied under restrictive conditions using only 4 eq of $NaIO_4$. Apart from information pertaining to

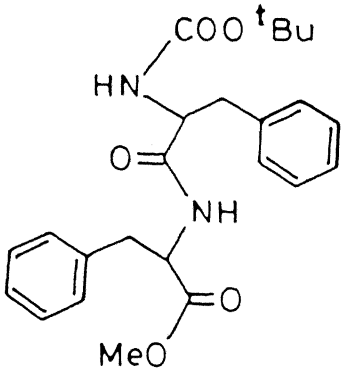
the susceptibility of the aromatic phenyl ring, the possibility existed that a preference may even exist in such oxidations, depending on whether the desired side chain is at the N-terminal end. For example, in the event compound (21) underwent prefential oxidation of the Phe side chain from the amino end, the product would be BocAsp-PheOMe, which could readily be transformed to Asp-PheOMe, aspartame, a sweetening agent used very widely. In the event, however, the reaction of (21) under conditions described above, led to the recovery of the starting material (CHART C.XXX).

A rather surprising result was the transformation of BzTyr-PheOMe (22) with Ru^{VIII} under normal conditions and for 12 h duration. The preferential oxidation of the tyrosine residue was not achieved. Instead, the product that was isolated in 74% yield was BzAsp-AspOMe (44). In spite of this, the lack of reaction in the case of (21) and other results (vide infra), strongly indicate that a preference for oxidation of the tyrosine side chain over that of phenylalanine could be accomplished provided the correct reaction conditions are worked out (CHART C.XXXVIII).

To date, the most fruitful results relating to studies on preferential oxidations have been obtained using tryptophan containing peptides as substrates.

The reaction of the dipeptide BzTrp-LeuOMe (23) with Ru^{VIII} under normal reaction conditions led to the isolation of BzAsp-LeuOMe (45) in 58% yields. This transformation is significant in the sense that it involves a photoactive and hydrophobic residue which is transformed to a polar acidic product lacking a chromophore. In addition, the transformation of the tryptophan residue to that of aspartic acid opens numerous possibilities since a variety of modified peptides can arise from (45) by taking advantage of the highly

CHART C-XXXVII



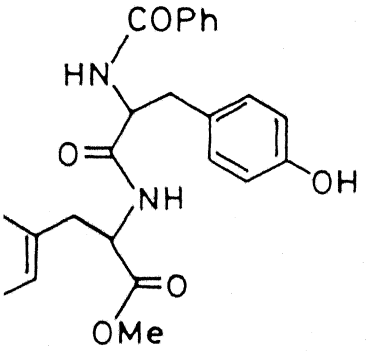
a → No reaction

Boc Phe–PheOMe

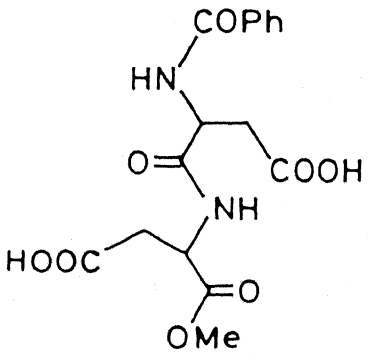
(21)

a: $RuCl_3 \cdot 3H_2O$ – $NaIO_4$ (4 eq), MeCN–CCl_4–H_2O, 60 h, rt

CHART C-XXXVIII



a →



Bz-Tyr-Phe-OMe (22) Bz-Asp-Asp-OMe (44)

$RuCl_3 \cdot 3H_2O$ – $NaIO_4$ (18 eq), MeCN–CCl_4–H_2O, 12 h, rt

versatile carbonyl grouping (CHART C.XXXIX). The expected preferential oxidation of the tryptophan residue over that of phenylalanine was accomplished in good yields in the reaction of BzTrp-PheOMe (24) with Ru^{VIII} using 18 eq of periodate but under a restricted duration of 8 h of reaction (CHART C.XXXIX). Careful analysis of the reaction mixture gave, as the neutral product, N^{α} -Bz N^{ω} -ForKyn-PheOMe (46, 13%) possessing a non-coded side chain in addition to BzAsp-PheOMe (47), which was isolated in 66% yields. The isolation of (46) illustrates the versatility of the chemical approach at site specific modification leading to products that could not be obtained by genetic engineering. BzAsp-PheOMe, (47), is N-protected aspartame and, indeed, similar oxidations with BocTrp-PheOMe or even Trp-PheOMe at low pH would offer a one-flask route to aspartame. The advantage of this approach to the aspartyl phenylalanine dipeptide is that it obviates the need for the removal of the β -protecting group which, generally, is not a very efficient process[52].

N^{α} -Bz N^{ω} -ForKyn-PheOMe (46) :

mp. 181 - 183°C

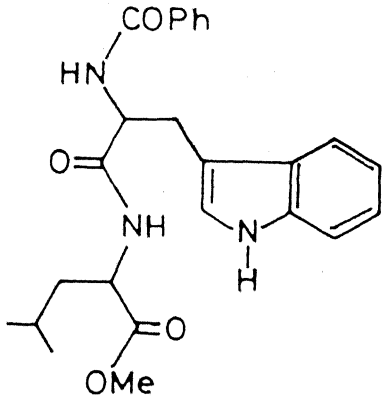
ir : ν_{max}(KBr) cm^{-1} : 3260 (-NH), 1730 (ester), 1630, 1520 (amide).

nmr : δ ($CDCl_3$) : 3.1 (d, 2H, -CHC$\underline{H_2}$Ph), 3.7 (m, 5H, -CHC$\underline{H_2}$CO, -COOC$\underline{H_3}$), 4.8 (br, 1H, -C$\underline{H}$), 5.2 (br, 1H, -C$\underline{H}$), 6.8 - 7.9 (m, 15H, -N$\underline{H}$, aromatic protons), 8.3-8.8 (br, 2H, PhCON$\underline{H}$, -N$\underline{H}$CHO).

ms : m/z : 501 (M^+), 502 (M^++1).

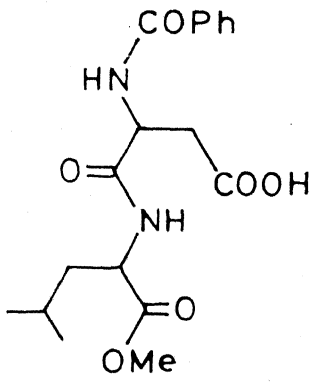
$[\alpha]_D^{25}$: +72.82 (c, 0.46 ; $CHCl_3$)

CHART C-XXXIX



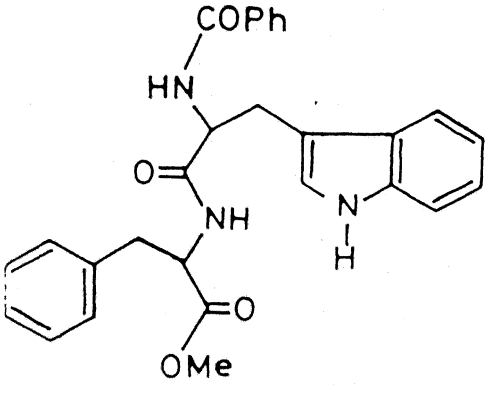
Bz—Trp—Leu—OMe

(23)



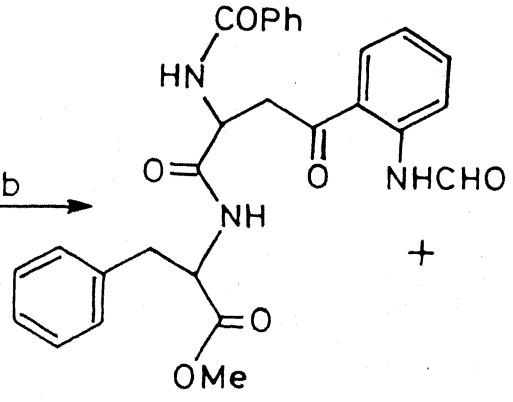
Bz—Asp—Leu—OMe

(45)



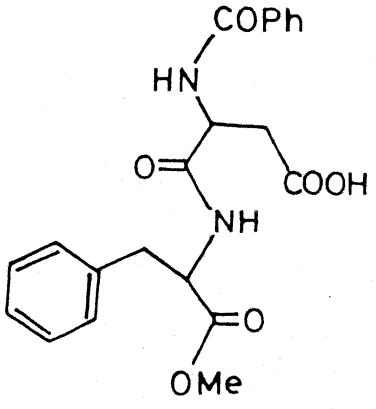
Bz-Trp-Phe-OMe

(24)



N^{α}-Bz-N^{ω}-For-Kyn-Phe-OMe

(46)



Bz-Asp-Phe-OMe

(47)

a : $RuCl_3.3H_2O - NaIO_4$ (18 eq), MeCN - CCl_4 - H_2O, 60h, rt

b : Same as 'a', 8h, rt

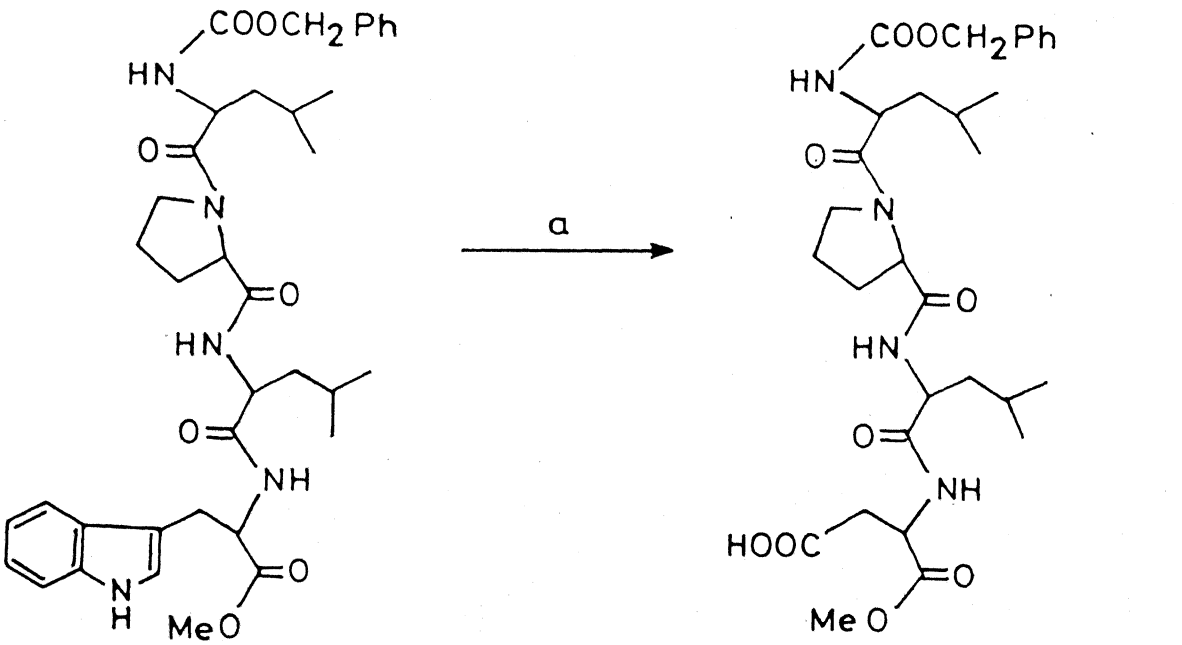
The confidence with which the tryptophan residue in peptides could be chemically transformed to that of aspartic acid finds an excellent and attractive illustration in the transformation of the tetrapeptide ZLeu-Pro-Leu-TrpOMe (51). This compound, with 18 eq of $NaIO_4$, is transformed with Ru^{VIII}, during a short period of 8 h, cleanly, to ZLeu-Pro-Leu-AspOMe (52) in 60% yields and without affecting the proline residue. The structure of (52) was confirmed by amino acid analysis and matching the found residues with that anticipated (CHART C.XL).

The susceptibility of the proline residue towards oxidation with RuO_4 as a function of its placement in a peptide was illustrated using the dipeptide pair BzPro-PheOMe (25) and BzPhe-ProOMe (26). It was anticipated that in the case of (25) where the N-benzoyl protecting group is directly attached to the proline residue, its proclivity for oxidation would be higher compared to (26) where the proline group is in an environment that is comparable to that present in a peptide such as (51) and others (vide infra). This expectation was, happily, realized. The reaction of (25) under the usual conditions, gave, the expected BzPro-AspOMe (49, 29%) and the oxidized product BzGlu-AspOMe (48, 14%), both of which were identified after esterification with CH_2N_2 and comparison with authentic samples (30) and (31) respectively. In striking contrast, BzPhe-ProOMe (26), under similar conditions, gave, exclusively, in 62% yield, BzAsp-ProOMe (50), identified by conversion into its dimethyl ester, BzAsp(β -OMe)-ProOMe. A careful analysis of the reaction mixture could not lead to isolation of products where the proline ring was affected (CHART C.XLI).

BzAsp(β -OMe)-ProOMe :

Gummy solid

CHART C-XL

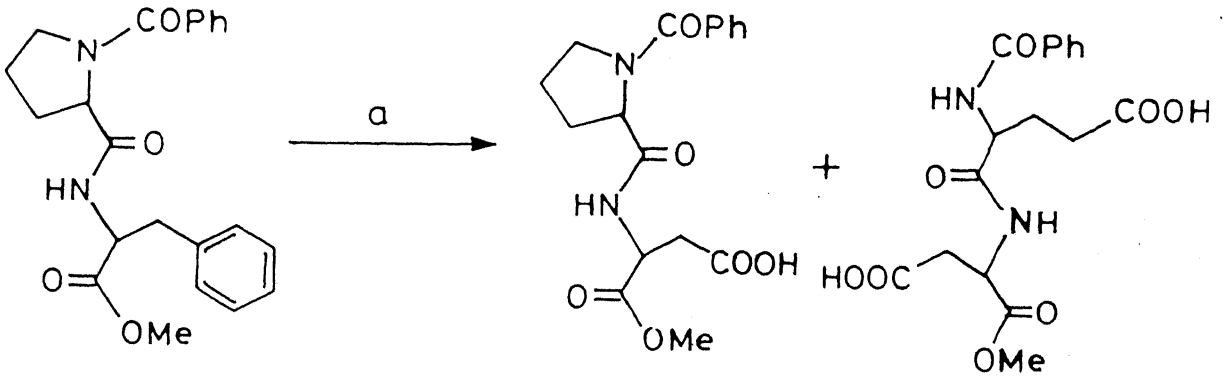


Z Leu - Pro - Leu - Trp OMe
(51)

Z Leu - Pro - Leu - Asp OMe
(52)

a : $RuCl_3 \cdot 3H_2O$ - $NaIO_4$ (18 eq), MeCN - CCl_4 - H_2O, 8h, rt

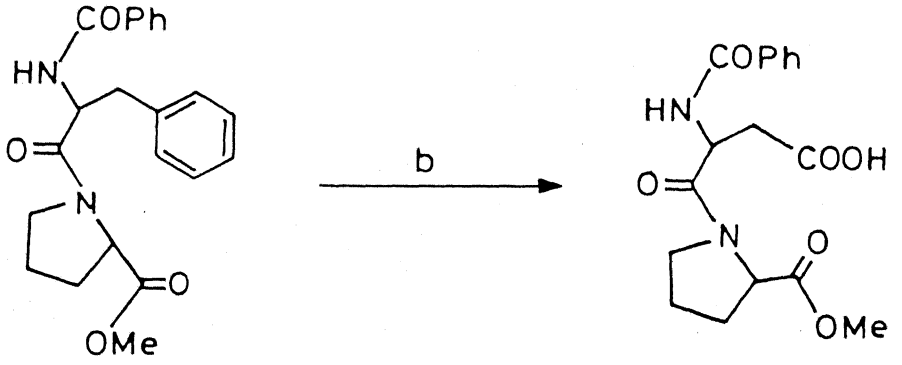
CHART C-XLI



Bz-Pro-Phe-OMe (25)

Bz-Pro-Asp-OMe (49)

Bz-Glu-Asp-OMe (48)



Bz-Phe-Pro-OMe (26)

Bz-Asp-Pro-OMe (50)

a: $RuCl_3.3H_2O - NaIO_4$ (18 eq), $MeCN - CCl_4 - H_2O$, 12 h, rt

b: Same as 'a', 8 h, rt

ir : ν_{max}(neat) cm^{-1} : 3300 (-NH), 1725 (ester), 1635, 1525 (amide).

nmr : δ ($CDCl_3$) : 1.5 - 2.45 (m, 4H, -CH($\underline{CH_2}$)$_2$), 2.75 (d, 2H, -CH$\underline{CH_2}$Ph), 3.1 - 4.1 (m, 8H, 2 x -COO$\underline{CH_3}$, -(CH_2)$_2$$\underline{CH_2}$N), 4.35 (m, 1H, -$\underline{CH}$), 5.3 (m, 1H, -$\underline{CH}$), 6.5 (br, 1H, -$\underline{NH}$), 7.0 - 8.1 (m, 5H, aromatic).

ms : m/z : 362 (M^+), 363 (M^++1).

The oxidative transformations of dipeptides and a tetrapeptide carried out thus far is presented in TABLE C-II. Studies outlined in TABLE C-I gave an indication of possible preferences in side chain oxidation which, to a large measure, have been substantiated as shown in TABLE C-II. In TABLE C-III is presented selectivity anticipated and observed in Ru^{VIII}- mediated oxidations a highly satisfying aspect of which is that even with the very limited amount of experimental results that have been obtained, it is possible to define conditions where a very high degree of selectivity could be obtained.

D. The chemoselective oxidation of the hydrophobic region of c - lysozyme :

The mechanisms by which the hydrophobic regions of proteins are recognized by membranes are domains under intense current scrutiny[60]. A problem frequently encountered with such studies is the difficulty in attaching the appropriate membrane probes[61]. The preceeding account of the present work has illustrated the transformation of a number of aromatic side chains to that of aspartic acid. As stated earlier, this residue offers numerous possibilities for further modification, one of which would be the attachment of ligands that would serve as effective membrane probes. This approach is

TABLE C-II

S.No.	SUBSTRATE	REACTION TIME (h)	PRODUCT/S	YIELD
1	Boc Phe - Phe OMe (21)[a]	60	No change	-
2	Bz Tyr - Phe OMe (22)[b]	12	Bz Asp - Asp OMe (44)	74
3	Bz Trp - Leu OMe (23)[b]	60	Bz Asp - Leu OMe (45)	58
4	Bz Trp - Phe OMe (24)[b]	12	N^{α} - Bz N^{ω} - For Kyn - Phe OMe (46) Bz Asp - Phe OMe (47)	13 66
5	Z Leu - Pro - Leu - Trp OMe (51)[b]	12	Z Leu - Pro - Leu - Asp OMe (52)	60
6	Bz Pro - Phe OMe (25)[b]	12	Bz Glu - Asp OMe (48) Bz Pro - Asp OMe (49)	14 29
7	Bz Phe - Pro OMe (26)[b]	12	Bz Asp - Pro OMe (50)	62

"a" : MeCN : CCl_4 : H_2O :: 4 : 4 : 8 ml , $NaIO_4$ (4 mmol) / m mol of substrate ; $RuCl_3 \cdot 3H_2O$ (2.2 mol %) at rt.

"b" : $NaIO_4$ (18 mmol) used in "a" above .

TABLE C-III

	SELECTIVITY IN RUTHENIUM TETROXIDE OXIDATIONS							
	PHE	TYR	TRP	HIS	LYS	PRO	MET	CYS
15 eq periodate, 60h, pH5.5	+	+	+	+	?	poor	+	+
8 eq periodate, 8h, pH 5.5	-	+	+	?	?	-	+	+
8 eq periodate, 0.5h, pH 5.5	-	-	+	?	-	-	+	+
8 eq periodate, 0.5h, pH 3	-	-	+	-	-	-	+	+

particularly relevant in the case of signal peptides, transient N - terminal sequences 15 - 20 residues long, which are essential for proteins to be targeted to the appropriate membrane sites and translocation across these membranes. Extensive studies have shown that the recognition by the hydrophobic middle part of the signal peptide is crucial for the transfer, although the nature of the interactions involved have not yet become clear. Whilst it is accepted that signal peptide - lipid interactions contribute significantly to lower the energy barrier to protein translocation, it remains to be clearly established whether this interaction is directly associated with the membrane[62], or via the intermediacy of the signal recognition particle[63].

The possibility of transforming an appropriate residue in one of the signal sequences to a carboxyl group appeared quite attractive, since, as stated earlier, such a transformation would enable the attachment of appropriate membrane probes that would be valuable in the study of the interaction of signal sequences with suitable receptors. The Cys-10 deleted hydrophobic region of c - lysozyme, namely, the undecapeptide BocLeu-Val-Leu-Phe-Leu-Pro-Leu-Ala-Ala-Leu-GlyOBzl (53) was chosen as an attractive target for several reasons. Recent work has shown that replacement of the Cys - 10 residue with Leu - 10 increases the secretion of h - lysozyme significantly without changing the processing site, thus indicating that Cys - 10 has no specific role[64]. In addition, the undecapeptide sequence consists of a single phenylalanine residue, which, if transformed to that of aspartic acid, would provide the required handle for attachment of membrane probes. Another exciting aspect of this undecapeptide is that it contains a proline residue and the reaction of the undecapeptide (53) with Ru^{VIII} would provide a realistic test pertaining to capabilities of bringing about site specific changes in more complex peptides.

In the event, the chromatographically homogeneous signal hydrophobic segment (53), (100 mg), on treatment with Ru^{VIII} under normal conditions yielded, cleanly, the expected site altered undecapeptide (54) in quantitative yields. The structural assignment for (54) is supported by amino acid analysis and comparison with expected values. As anticipated from previous studies, the proline moiety being in a true peptide environment was not affected at all.

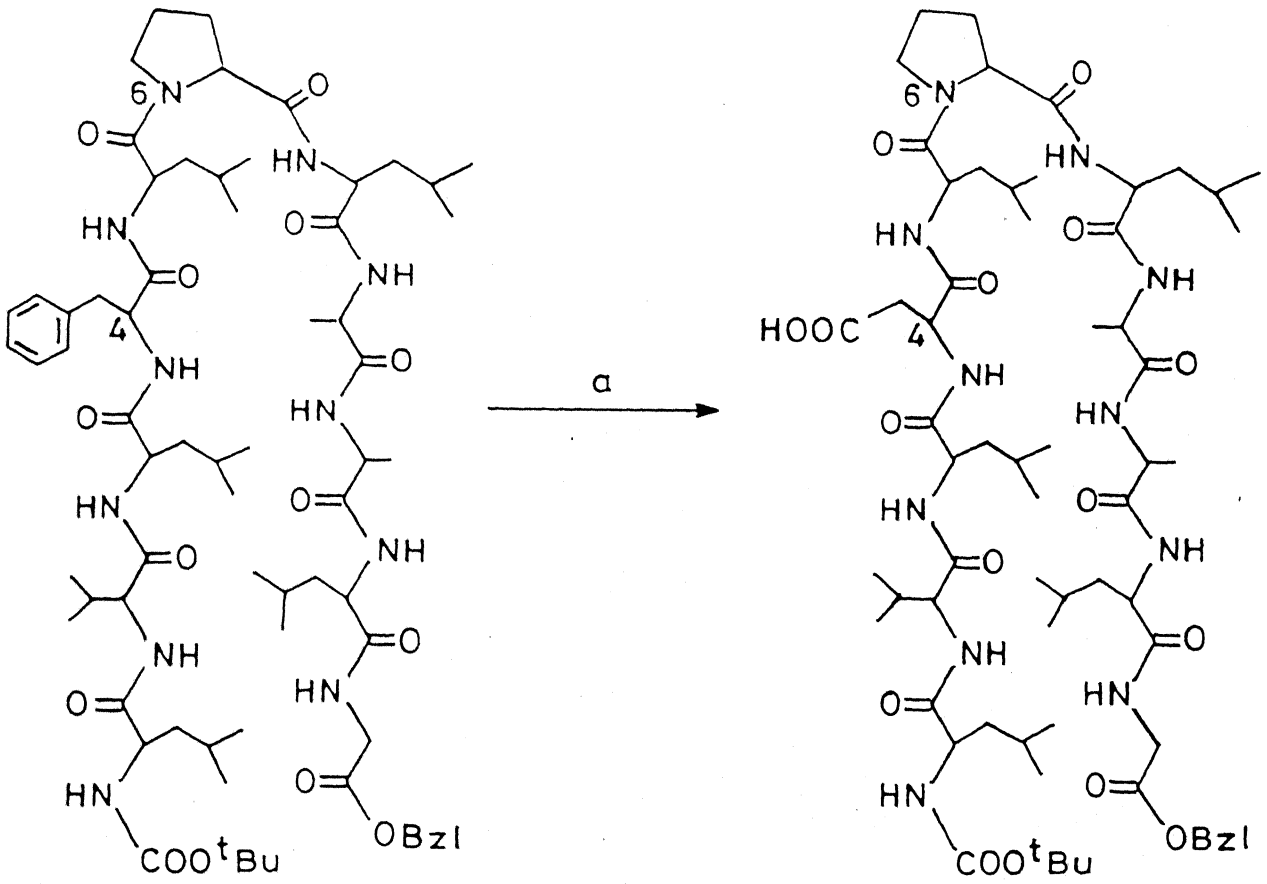
The (53) → (54) change, above all, represents a significant stage in the planned development of an oxidative methodology to bring about site specific side chain changes (CHART C.XLII).

E. The chemoselective oxidation of melittin :

Melittin, a protein containing 26 amino acid residues, is the principal toxic component of bee venom. It is water soluble as a tetramer, but it spontaneously integrates into lipid bilayers and is thought to act as a lytic agent. The choice of melittin to demonstrate site selective transformation largely stems from the fact that it contains a single tryptophan residue at the 19-position and, therefore, would be an attractive target for modification to that of an aspartic acid. An important point to note is that melittin, in addition, is endowed with a range of susceptible groups, namely, lysine, threonine, proline, serine and arginine and, in addition, has an unprotected amino group. Consequently, the site specific transformation of melittin can be considered as a realistic yet difficult exercise.

In the event, 2 mg of the polypeptide (55) was oxidized with Ru^{VIII} using 18 eq of periodate for a duration of 8 h. Product analysis including optical data and amino acid analysis clearly indicated the transformation

CHART C-XLII



(53)

Boc Leu – Val – Leu – ^{4}Phe – Leu –
^{6}Pro – Leu – Ala – Ala – Leu – Gly – OBzl

(54)

Boc Leu – Val – Leu – ^{4}Asp – Leu –
^{6}Pro – Leu – Ala – Ala – Leu – Gly – OBzl

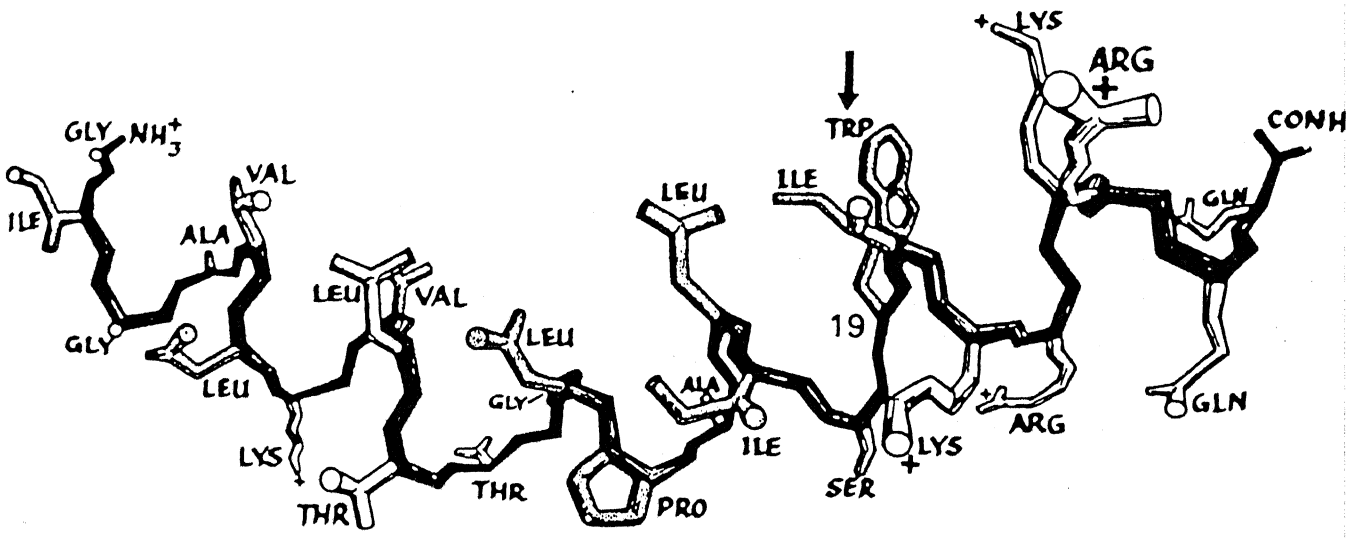
a: $RuCl_3 \cdot 3H_2O - NaIO_4$ (18 eq), MeCN – CCl_4 – H_2O, 60 h, rt

of the tryptophan to aspartic acid. The most important finding was that the transformed product exhibited an ORD pattern identical to the precursor, thus indicating that the oxidation did not alter the secondary structure of the protein nor did if effect ruputre of the peptide backbone (CHART C.XLIII)[65]. The corelation of the amino acids on the basis of an analysis with that expected was good excepting for proline and arginine where the paucity of material percluded the usage of the specialized analytical methods needed to detect these with accuracy.

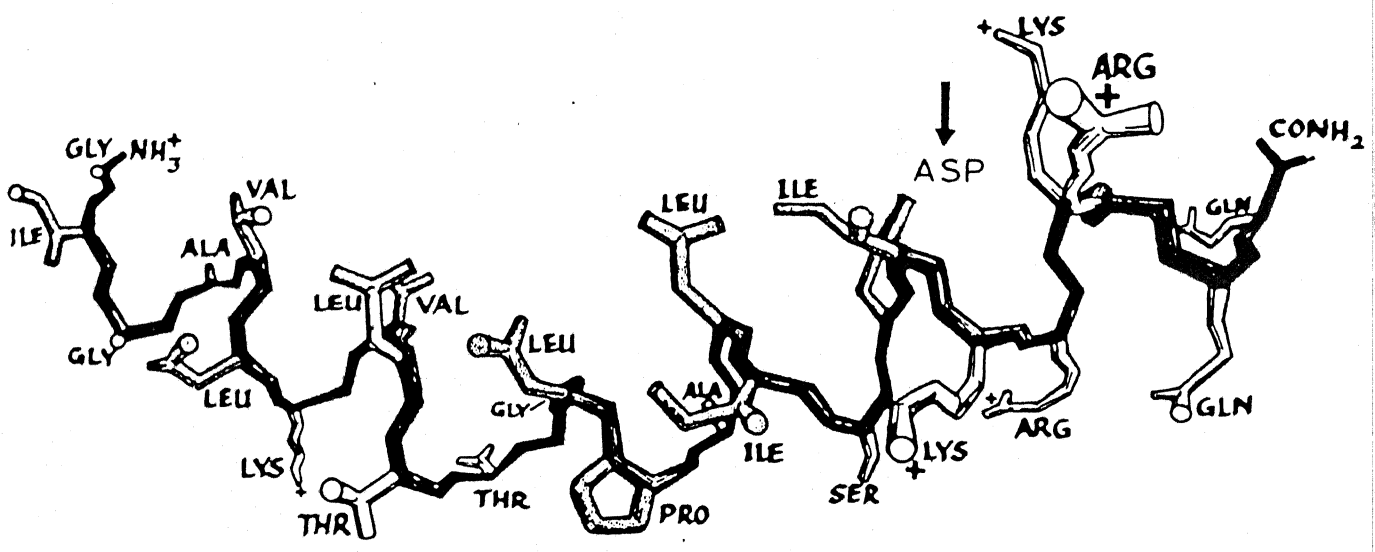
The (55)→ (56) change (CHART C.XLIV) further demonstrates possibilities of site specific side chain alterations by chemical methodologies.

The work described thus far was an account of endeavours directed at the development of a reliable methodology for site specific protein transformations. The results obtained have been satisfactory and confirm the belief that such methodologies could be developed and could find advantageous use in protein alteration in selected cases. The transformation of a hydrophobic residue to a carboxyl one or a methionine residue to the corresponding sulfone could have direct use if the damage of the enzyme concerned, is related to these two aspects. Indeed, one of the emerging areas in the domain of protein engineering is to effect selective side chain alteration such that a susceptible residue is eliminated. An illustration would be that of α_1-antitrypsin which prevents elastase activity in the lungs, implicated in the deactivation of which is the methionine residue[57]. Added incentive is that since 13 of the 20 coded amino acids are, in principle, susceptible to oxidation with Ru^{VIII}, the resulting products could, as cited above, find therapeutic applications, which would be the realization of one of the long range objectives of the present work.

CHART C-XLIII



(55)



(56)

CHART C-XLIV

MELITTIN

GLY – ILE – GLY – ALA – VAL – LEU – LYS – VAL – LEU – THR – THR –
GLY – LEU – PRO – ALA – LEU – ILE – SER – TRP – ILE – LYS – ARG –
19
LYS – ARG – GLN – GLN – NH_2

(55)

RuO_4

TRANSFORMED MELITTIN

GLY – ILE – GLY – ALA – VAL – LEU – LYS – VAL – LEU – THR – THR –
GLY – LEU – PRO – ALA – LEU – ILE – SER – ASP – ILE – LYS – ARG –
19
LYS – ARG – GLN – GLN – NH_2

(56)

F. Mechanistic studies on the oxidation of unprotected α-amino acids :

In the course of the elucidation of the mechanism pertaining to the transformation of the tryptophan side chain to that of aspartic acid, ancillary experiments were carried out using the free amino acids phenylalanine and valine, vide supra, leading to, respectively, phenylacetic acid and isobutyric acid. Thus, in the case of phenylalanine under the normal oxidation conditions, the α-amino moiety is preferentially oxidized. This finding was in contrast to that reported in literature, pertaining to the transformation of tyrosine at pH 3 using Ru^{VIII} species, giving rise to aspartic acid, which would amount to the preferential oxidation of the 4-hydroxyphenyl ring over the α-amino group present[66]. In addition, benzyl amine had been stated to yield glycine with Ru^{VIII} reagent, generated in situ, by hypochlorite[67]. In view of the detailed studies carried out on the oxidative transformations of coded α-amino acids outlined in an earlier section, it became important to delineate preferences for the side chain oxidations compared to that of the α-amino acid, and, at the same time, reconcile with the conflicting reports in the literature.

In competitive oxidations involving the side chain and the α-amino acid grouping, a factor that can play a key role would be the pH of the medium, in the sense that at very low pH ranges such as in phosphate buffer (pH~ 3), the amino group would be largely protonated and would therefore not be available for oxidation. Thus, the results obtained by us at pH 6 with phenylalanine leading to phenylacetic acid with those reported on tyrosine at pH 3 leading to aspartic acid could be reconciled by a study of these oxidations at various pH values. Additionally, the coded amino acids lysine and arginine possess two basic units of differing pka values. It was envisaged that the pH of the medium could be effectively used to control the oxidation of one

of these amino groups selectively over the other.

At the outset it was decided to perform the oxidation of the selected amino acids at three pH values, namely, in phosphate buffer at pH~ 3, in aqueous media (pH~ 6), and in saturated bicarbonate (pH~ 9).

The reaction of tyrosine with Ru^{VIII} in presence of 18 eq of $NaIO_4$ using MeCN-CCl_4-phosphate buffer (pH~ 3) as the media for 0.8 h at rt gave a 50% yield of aspartic acid confirming earlier reports. However, at pH 6 and 9, the exclusive product was malonic acid obtained, respectively, in 20 and 48% yields. Thus, through the entire range of pH under study, the p-hydroxyphenyl is always oxidized and the isolation of malonic acid shows that at higher pH values, the reaction pathway was precisely like that encountered in the transformation of phenylalanine at pH 6, namely, the prior oxidation of an α-amino acid to a keto acid, followed by further oxidation[54].

The importance of diverse dissociation equilibria that are latent in the switch over mechanism operating in the oxidation of tyrosine surfaces when the substrate is changed to phenylalanine, which, under neutral conditions, yields phenylacetic acid in 43% yields (vide supra). Of particular relevance was the observation that even at pH 3, conditions under which tyrosine exclusively yielded aspartic acid, phenylalanine gave a 76% yield of phenylacetic acid. Careful analysis of the reaction mixture revealed the complete absence of aspartic acid. This remarkable observation clearly demonstrates that even at pH 3, the amino acid moiety could undergo oxidation. This could be accomplished only on the basis of equilibria with the undissociated amine, although such an equilibrium can be quite unfavourable at a very low pH such as that under which the investigations were carried out. A comparison of the yields

obtained on oxidation of phenylalanine at pH 6 (43%) and pH 3 (76%) is noteworthy. At the higher pH range, benzoic acid is isolated as a product in about 13% yields, whose genesis could be understood on the basis of elimination followed by oxidation of the resulting cinnamic acid. It appears that a lower pH value discourages such an elimination and, consequently, the yield of phenylacetic acid is substantially higher in the phosphate buffer at pH 3.

The results obtained from tyrosine and phenylalanine at various pH values can be reconciled on the basis of pathways delineated in CHART C.XLV. At a low pH, the amino acid would be largely protonated and the only avenue for oxidation of this species is by involving the aromatic ring. On the other hand, equilibrium leading to dissociation to the free amino acid which would be substantial at pH 6 but extremely modest at pH 3, would lead to rapid transformation initiated by oxidation of the free amino group, as explained earlier, leading to aryl acetic acids. The aryl acetic acid, in turn, could be further oxidized in principle to malonic acid involving the aromatic moiety. With respect to tyrosine, at pH 3, the rate of oxidation of the electron rich p-hydroxyphenyl ring represented by k_1 in CHART C.XLV, is significant, leading to the formation of aspartic acid. Again, with this amino acid, at higher pH ranges, oxidation leads to the arylacetic acid, controlled by k_2 in CHART C.XLV, which is further oxidized to malonic acid. The substantially increased yield of malonic acid obtained at pH 9 should be a reflection of the availability of free amino group containing tyrosine, making the subsequent operations very facile. Phenylalanine offers an altogether different profile. At pH 3 in phosphate buffer under conditions of the reaction, the oxidation of the aromatic ring is negligible, and, therefore, the only available avenue for depletion of the substrate is via equilibrium with the free amino species, leading rapidly to the phenylacetic acid encountered earlier. The

CHART C-XLV

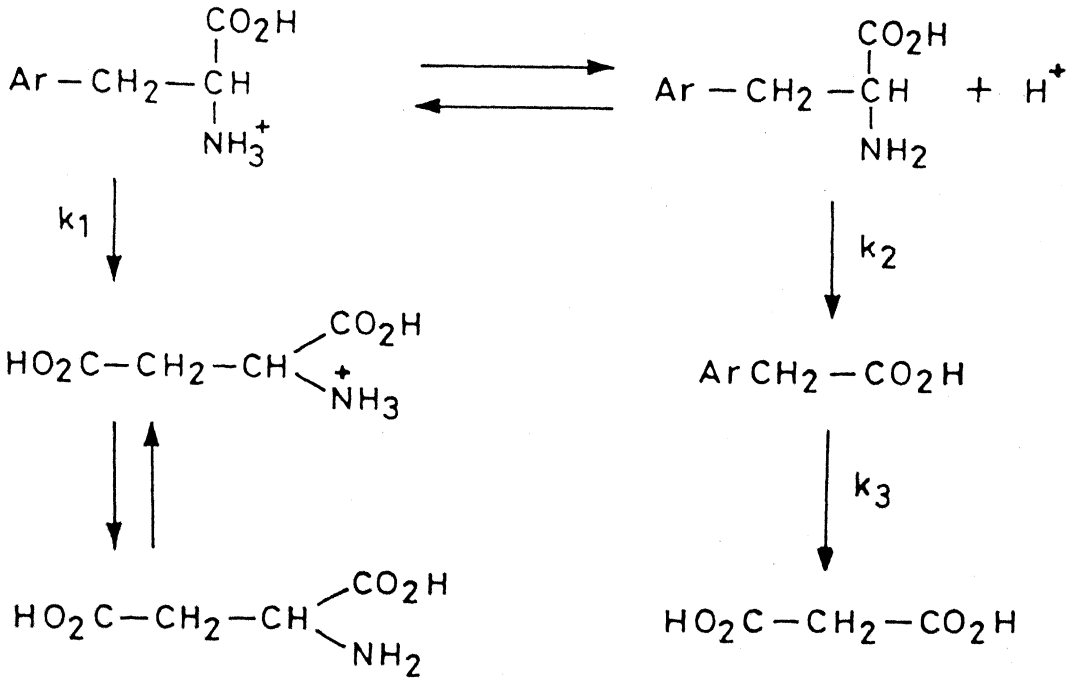
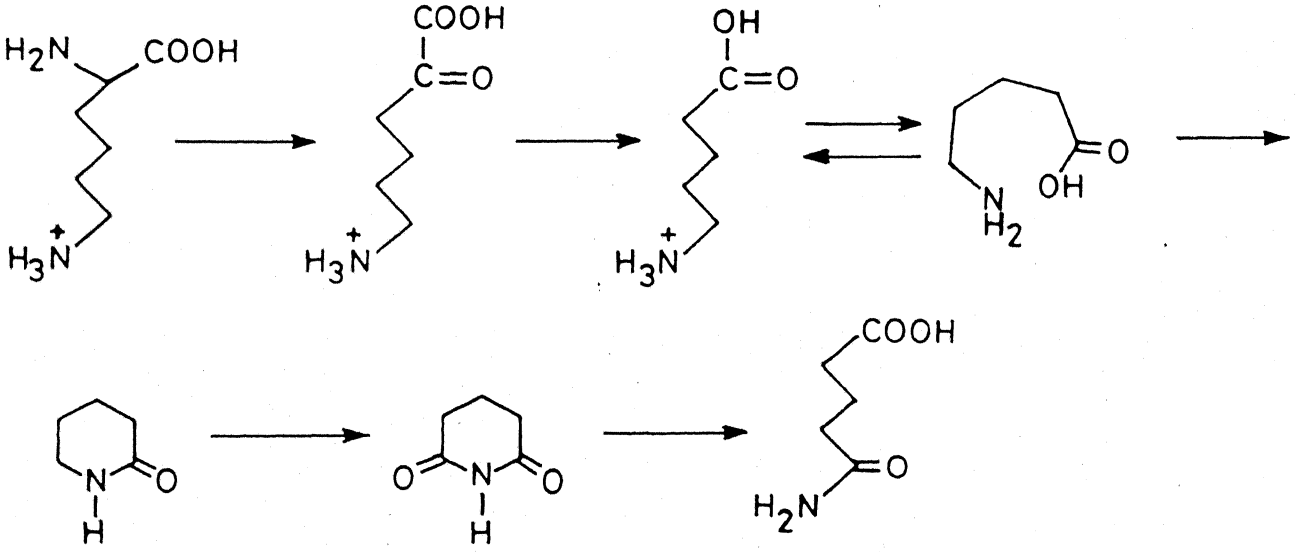


CHART C-XLVI



absence of formation of malonic acid in the case of phenylalanine at pH 3 as well as pH 6 implies that the aromatic ring is again very resistant to oxidation.

Attempts to transform benzyl amine to glycine, precisely under conditions reported[67], failed. This was not too surprising in view of comprehensive studies pertaining to the oxidation of benzyl amine with Ru^{VIII} [68]. Interestingly, oxidation of benzyl amine with Ru^{VIII} at pH 6 under the normal conditions, gave a complex mixture and the only crystalline product that could be obtained in 3% yield was benzyl benzamide, $PhCONHCH_2Ph$, thus demonstrating that to this extent at least the phenyl ring remains untouched.

The control of pH on the specificity of Ru^{VIII} mediated oxidations has been taken advantage of in demonstrating preferential oxidation using lysine as a substrate. The pronounced differences that exist between the pka of the α-amino group (pka 8.90), and the ω-amino function (pka 10.28), would suggest that the ω-amino group can be exclusively protonated. Consequently, oxidations could be restricted to the α-amino acid moiety. This was experimentally realized. Lysine, either at pH 3, or at pH 6, gave, as the sole isolable product, glutaric acid monoamide in, respectively, 34 and 33% yields. At a higher pH of 9 no pure product could be obtained. The lysine to glutaric acid monoamide change represents a remarkable reaction and the pathways involved in this change are rationalized in CHART C.XLVI. Both at pH 3 and 9 the ω-amino group of lysine would be largely protonated. Oxidation of the α-amino group then would lead to, via the α-keto acid, 5-amino pentanoic acid, which, on cyclization followed by further oxidation would lead to glutarimide. The oxidation of piperidone and similar compounds to the corresponding imides are known[53]. The opening of the glutarimide

would give rise to the mono amide of glutaric acid. The significance of this finding lies in the fact that at pH 3 certainly, and even at pH 6, lysine and arginine residues can be made insensitive to RuO_4 oxidations. The site specific oxidation of melittin (55) to (56) is an example of this understanding (vide supra).

$H_2NOC(CH_2)_3CO_2H$:

mp. 102°C

ir : ν_{max}(KBr) cm^{-1} : 3310 (-NH), 1685 (acid), 1620, 1520 (amide).

nmr : $\delta(CDCl_3)$: 1.8 (m, 2H, $-CH_2\underline{CH_2}CH_2$), 2.3 (m, 2H, $-CH_2\underline{CH_2}CONH_2$), 3.25 (m, 2H, $-\underline{CH_2}CO_2H$), 7.1 - 8.7 (br, 2H, $-CON\underline{H_2}$).

$H_2NOC(CH_2)_3CO_2Me$:

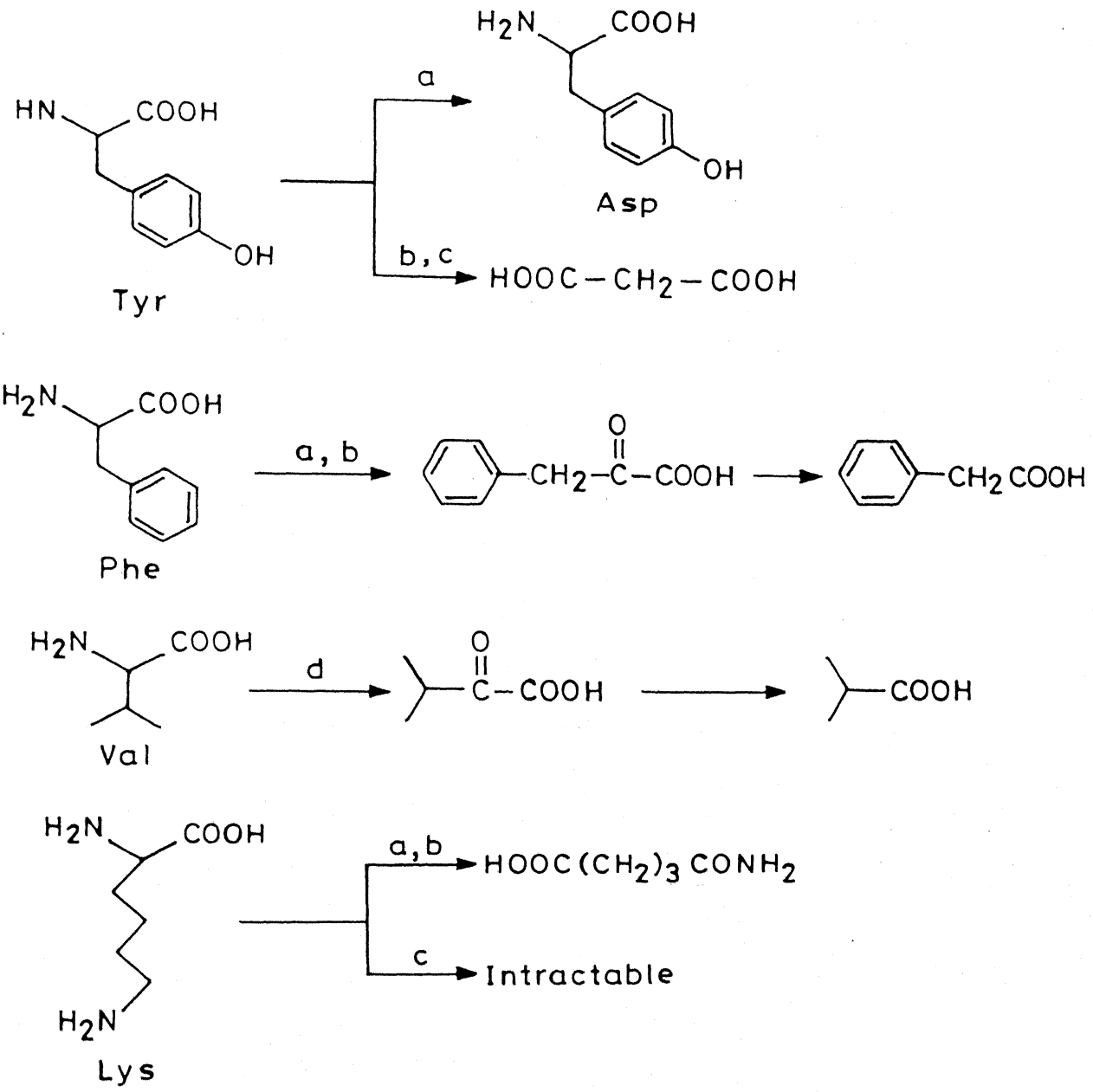
Oil

nmr : $\delta(CDCl_3)$: 1.9 (m, 2H, $-CH_2\underline{CH_2}CH_2$), 2.35 (t, 2H, $-\underline{CH_2}CONH_2$), 3.3 (m, 2H, $-\underline{CH_2}CO_2CH_3$), 3.65 (s, 3H, $-COOC\underline{H_3}$), 8.1 (br s, 2H, $-CON\underline{H_2}$).

ms : m/z : 145 (M^+)

The results obtained from studies on the free amino acids tyrosine, phenylalanine, valine and lysine are presented in CHART C.XLVII.

CHART C-XLVII



a: $RuCl_3.3H_2O - NaIO_4$ (18 eq), $MeCN - CCl_4$ – Phosphate buffer (pH~3), 0.8 h, rt

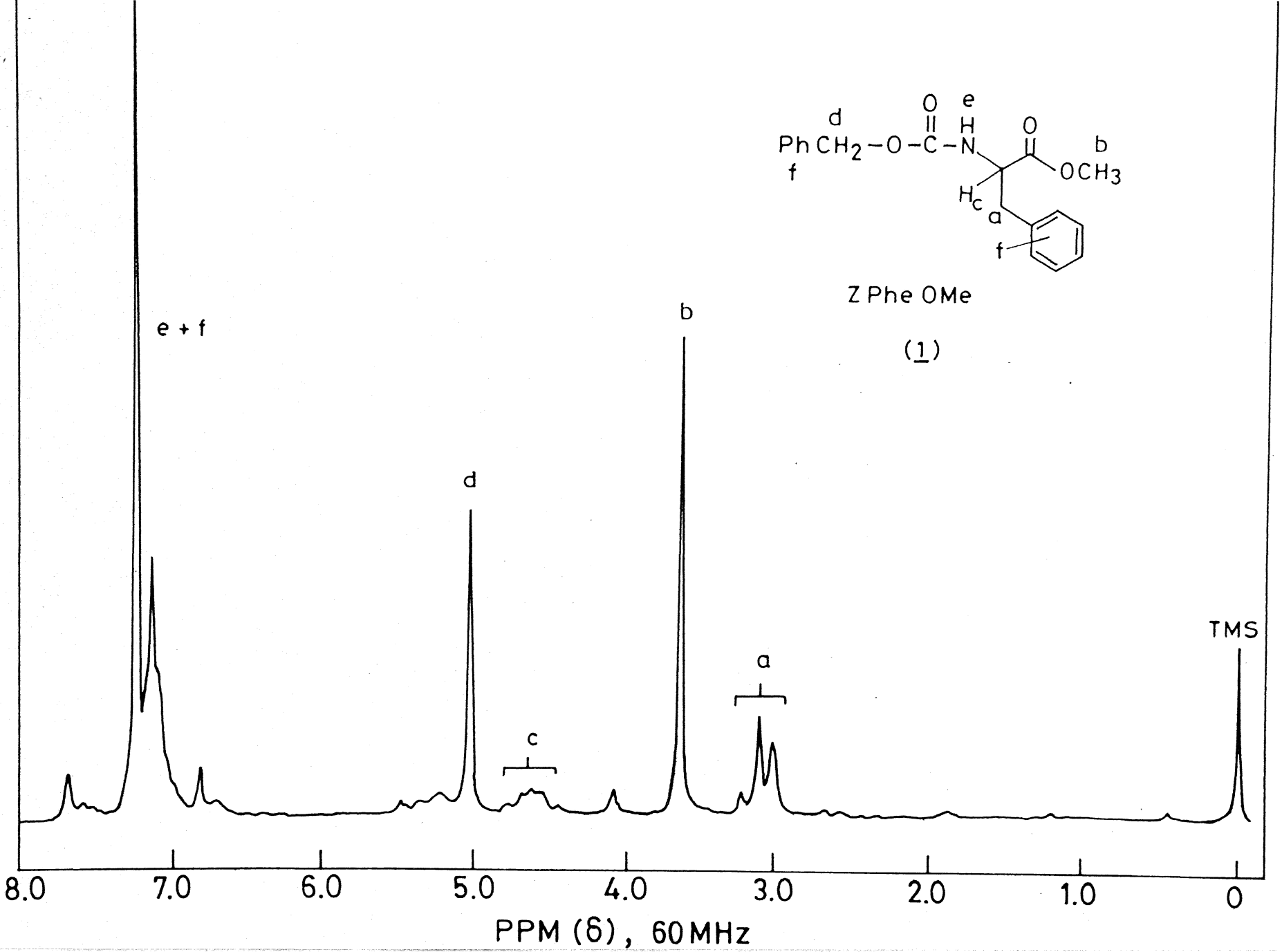
b: $MeCN - CCl_4 - H_2O$ used in 'a' above, 3 h, rt

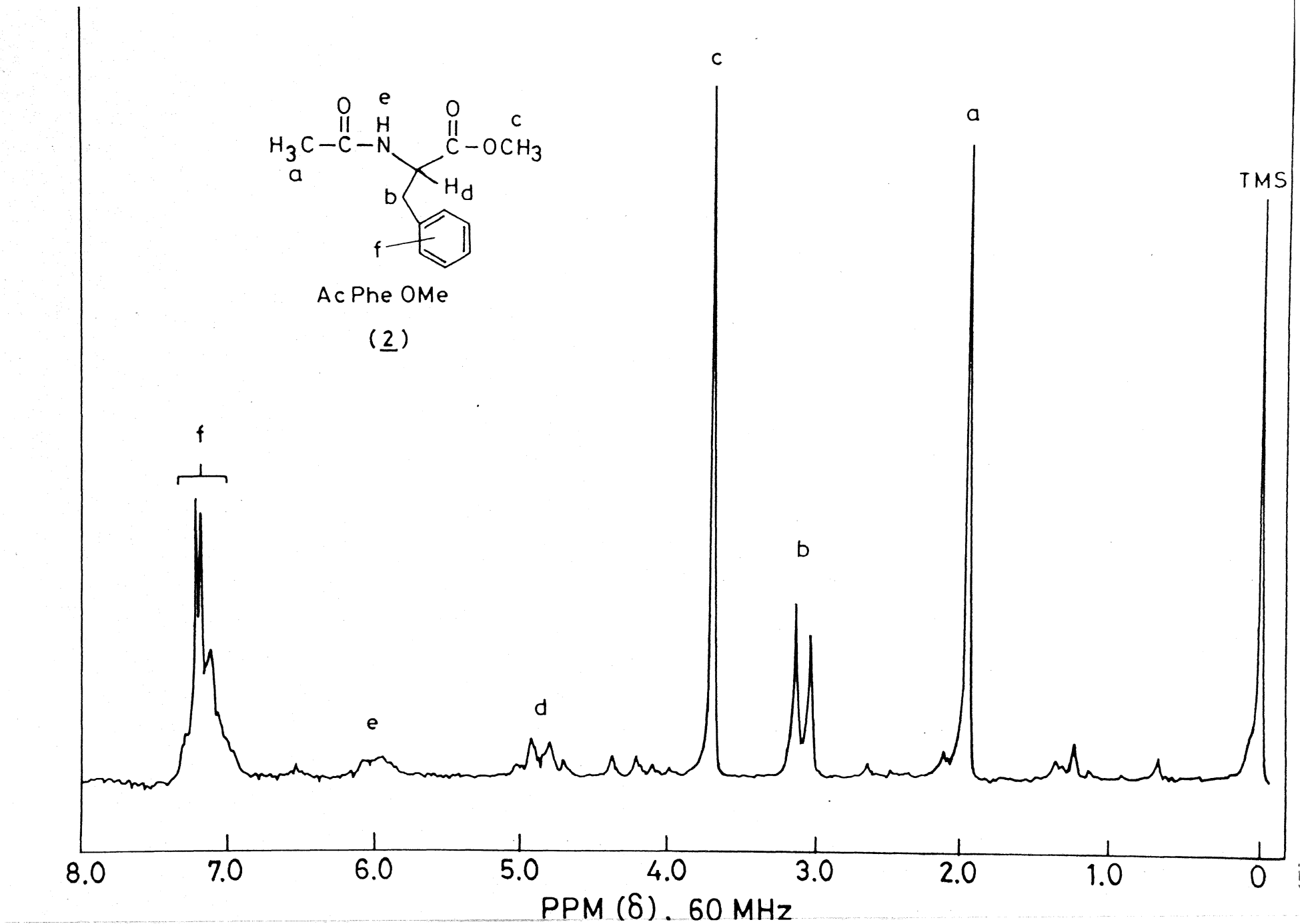
c: $MeCN - CCl_4$ – Satd. eq. $NaHCO_3$ (pH~9) used in 'a' above, 3h, rt

d: 'a', 60 h, rt

The reaction of either tryptophan or tryptophan methyl ester at pH 3, under normal reaction conditions, gave dark reaction mixtures from which no pure products could be isolated. In the case of histidine also, attempts to isolate pure compounds from Ru^{VIII} oxidations did not succeed.

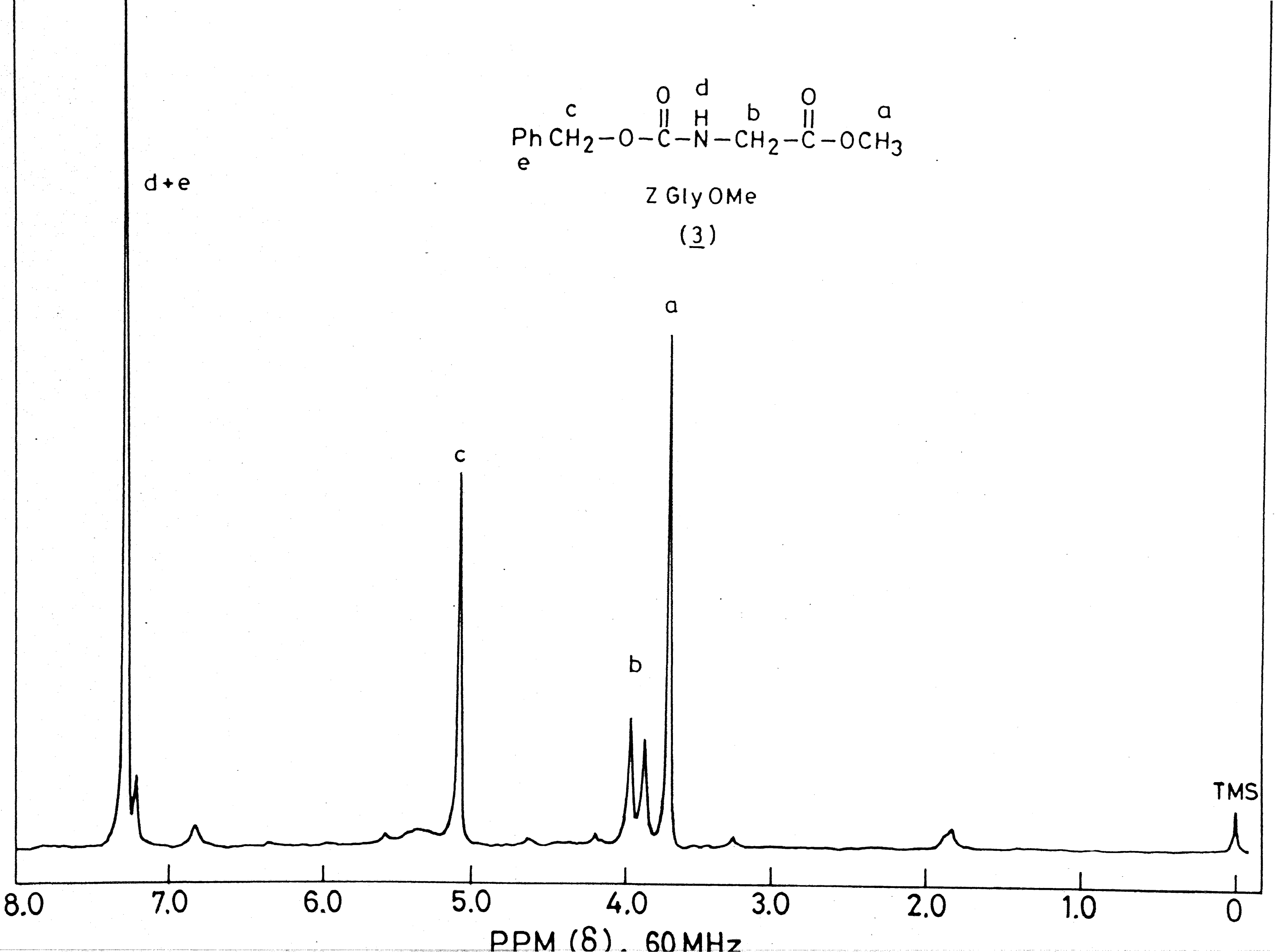
The above account summarizes studies, to date, pertaining to the oxidative transformations of amino acids using Ru^{VIII} reagent. The project was planned and developed in stages and has, hopefully, led to not only a deeper understanding of the behaviour of the oxidizing system towards diverse substrates, but also has provided confidence to the effect that chemically mediated site specific oxidative alterations can be performed even in complex substrates.


d
e
O
H
O
Ph CH2 – O – C – N
b
f
OCH3
Hc
a
f
Z Phe OMe
(1)
e + f
b
d
c
a
TMS
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ), 60 MHz

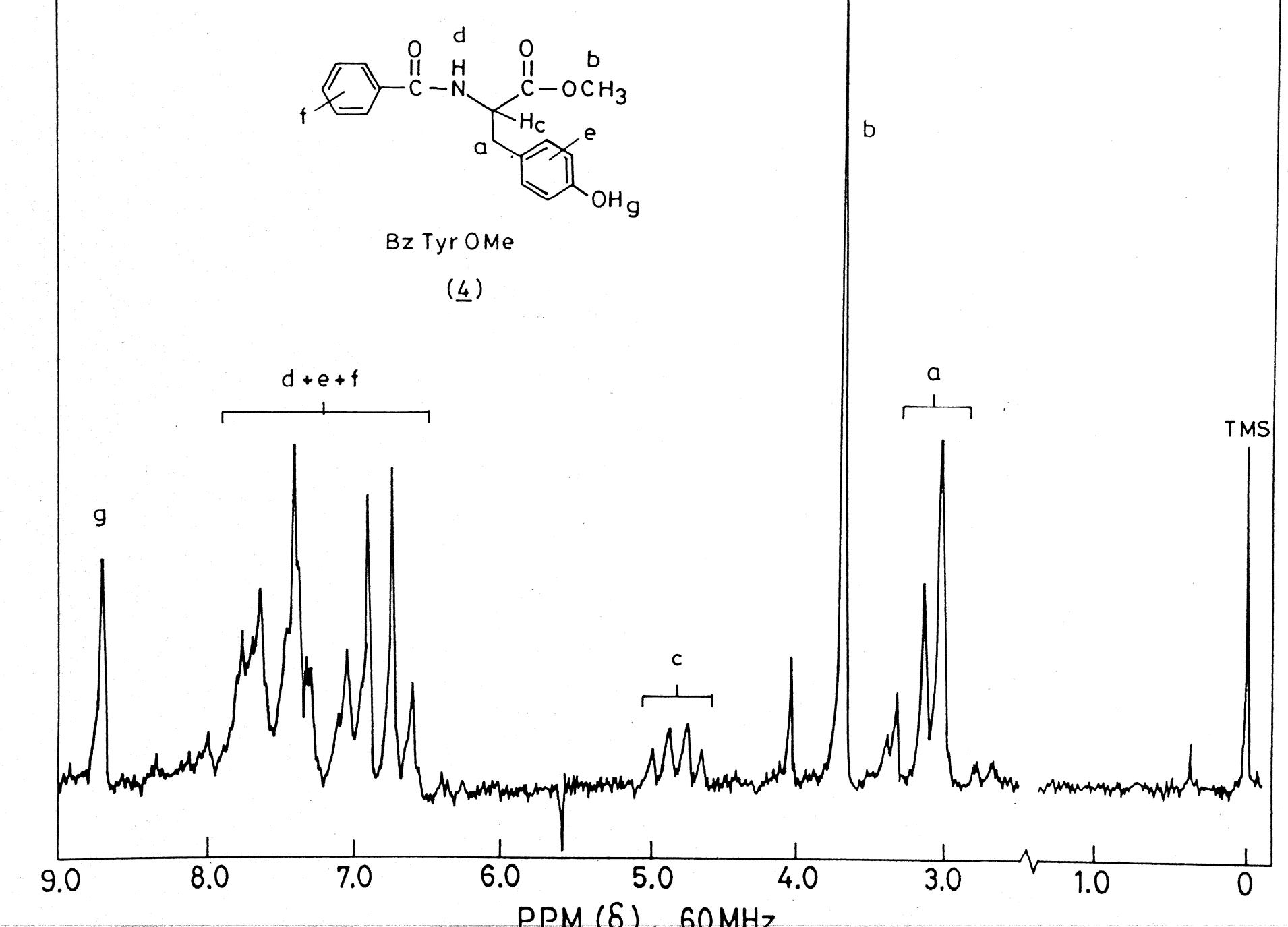


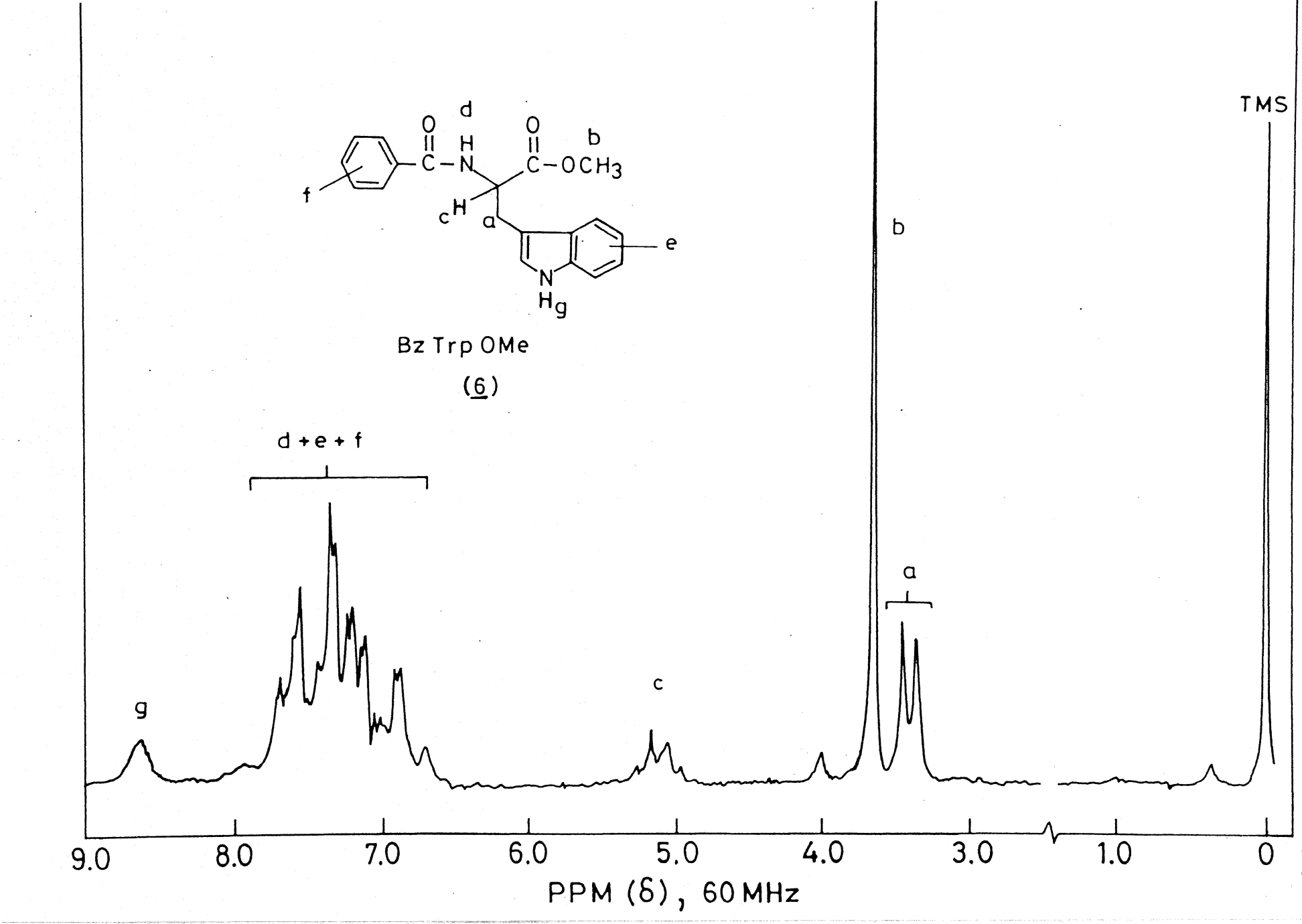
Ac Phe OMe

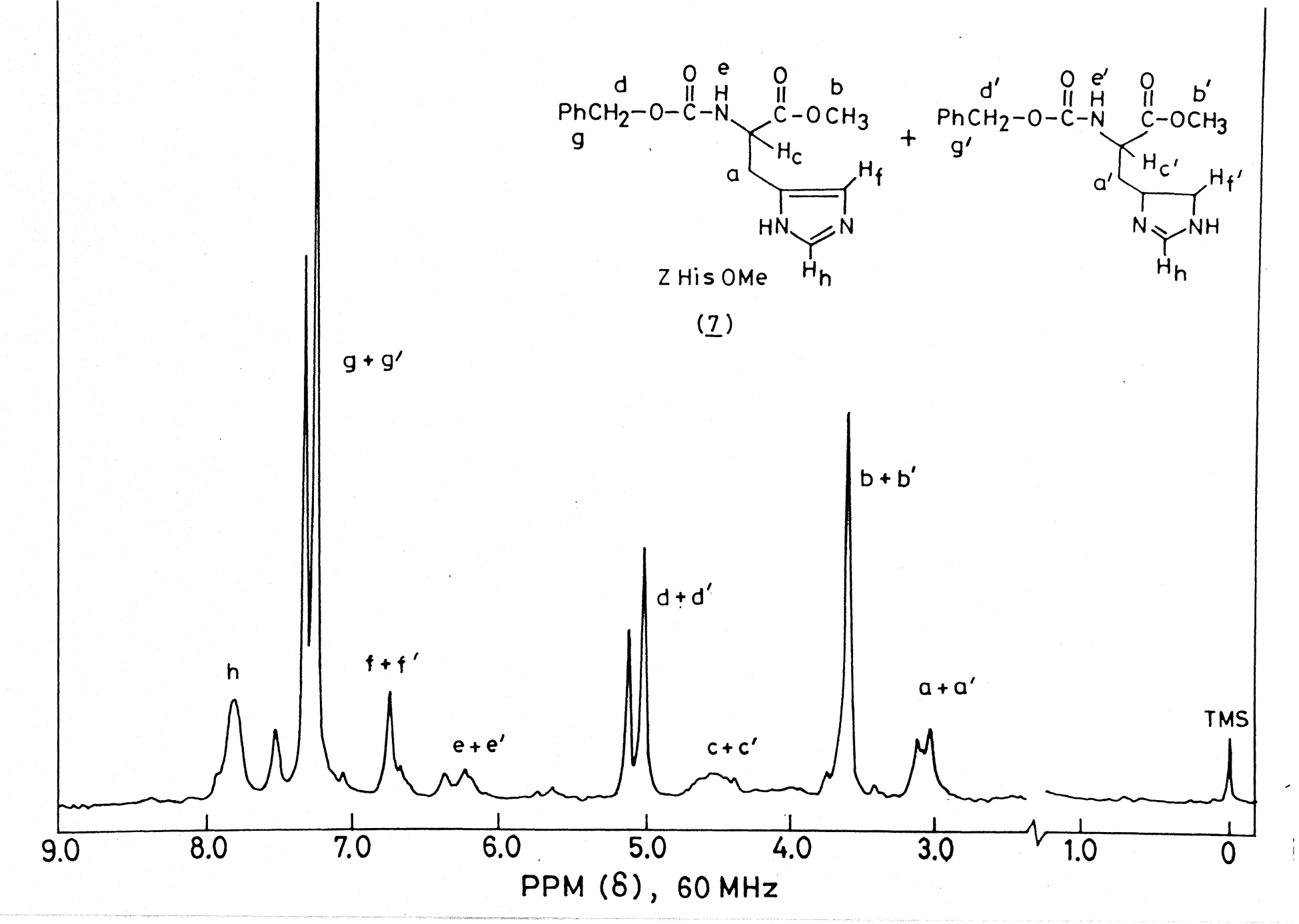
(2)

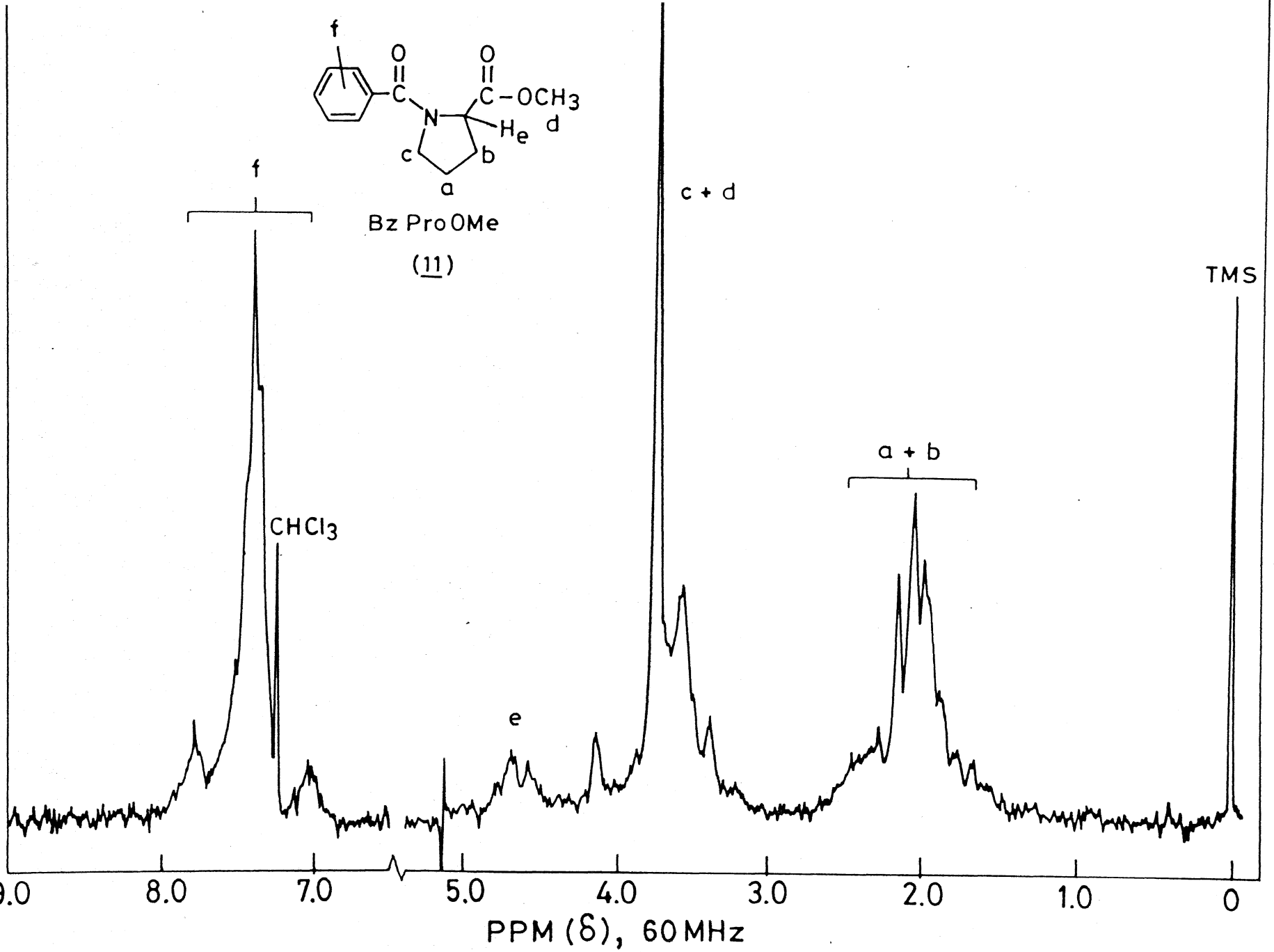


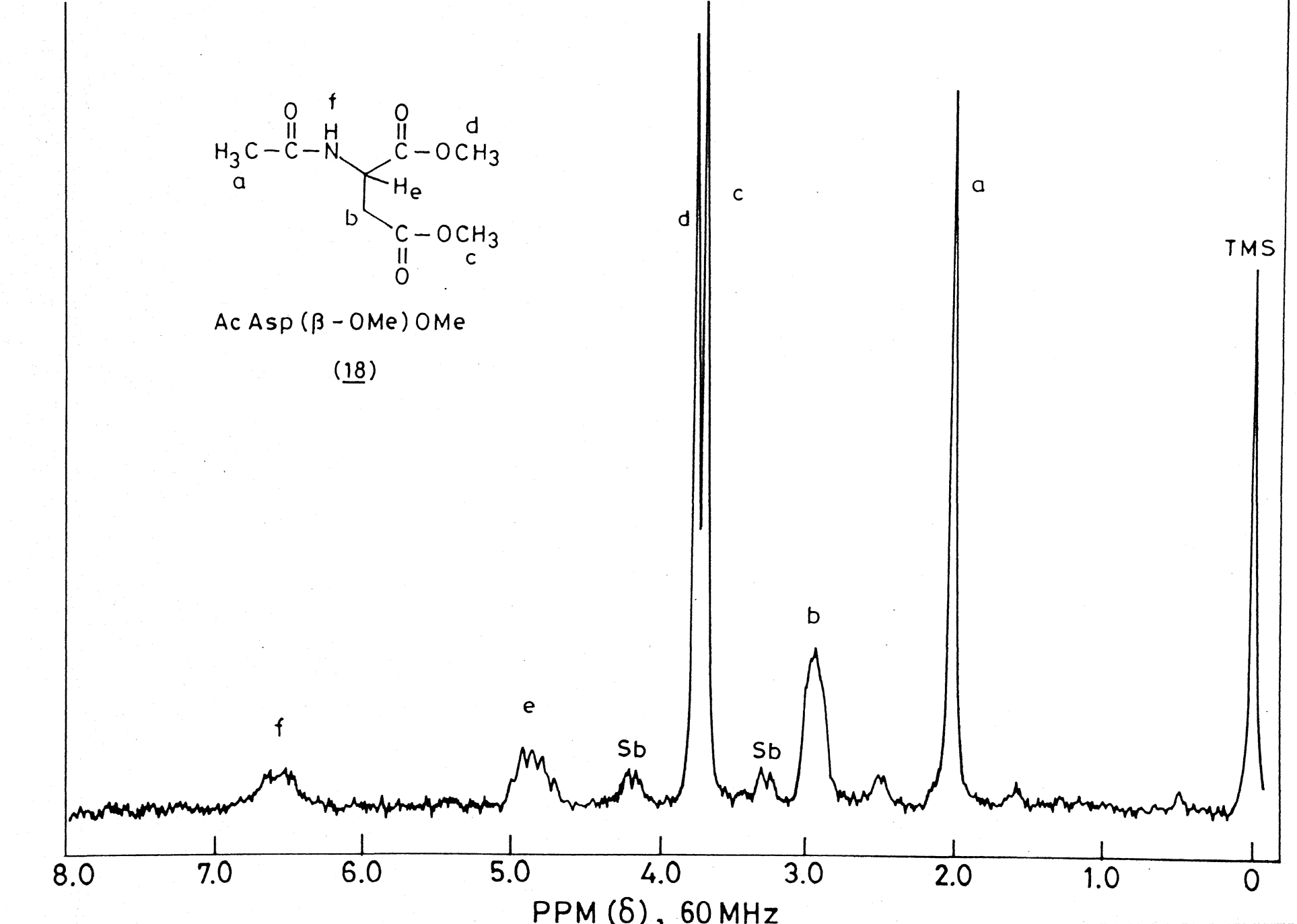
Ph CH2–O–C(=O)–N(H)–CH2–C(=O)–OCH3
e c d b a
Z Gly OMe
(3)
d+e
c
b
a
TMS
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ), 60 MHz

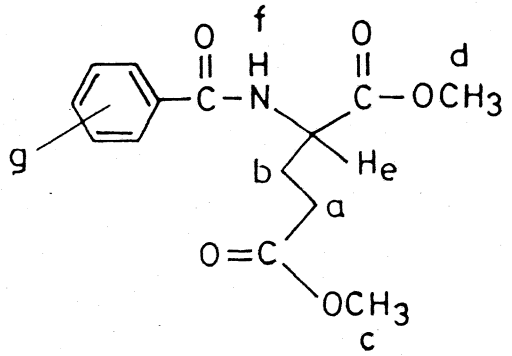

d
O
H
O
b
C – N
C – OCH3
f
Hc
a
e
OHg
b
Bz Tyr OMe
(4)
d + e + f
a
TMS
g
c
9.0
8.0
7.0
6.0
5.0
4.0
3.0
1.0
0
PPM (δ) 60MHz


d
O
H
O
b
C–N
C–OCH3
f
cH
a
e
N
Hg
Bz Trp OMe
(6)
d + e + f
g
c
b
a
TMS
9.0
8.0
7.0
6.0
5.0
4.0
3.0
1.0
0
PPM (δ), 60 MHz


d
e
b
PhCH2–O–C–N
C–OCH3
g
Hc
a
Hf
HN
N
Hh
Z His OMe
(7)
+
d′
e′
b′
PhCH2–O–C–N
C–OCH3
g′
Hc′
a′
Hf′
N
NH
Hh
g + g′
h
f + f′
e + e′
d + d′
c + c′
b + b′
a + a′
TMS
9.0
8.0
7.0
6.0
5.0
4.0
3.0
1.0
0
PPM (δ), 60 MHz

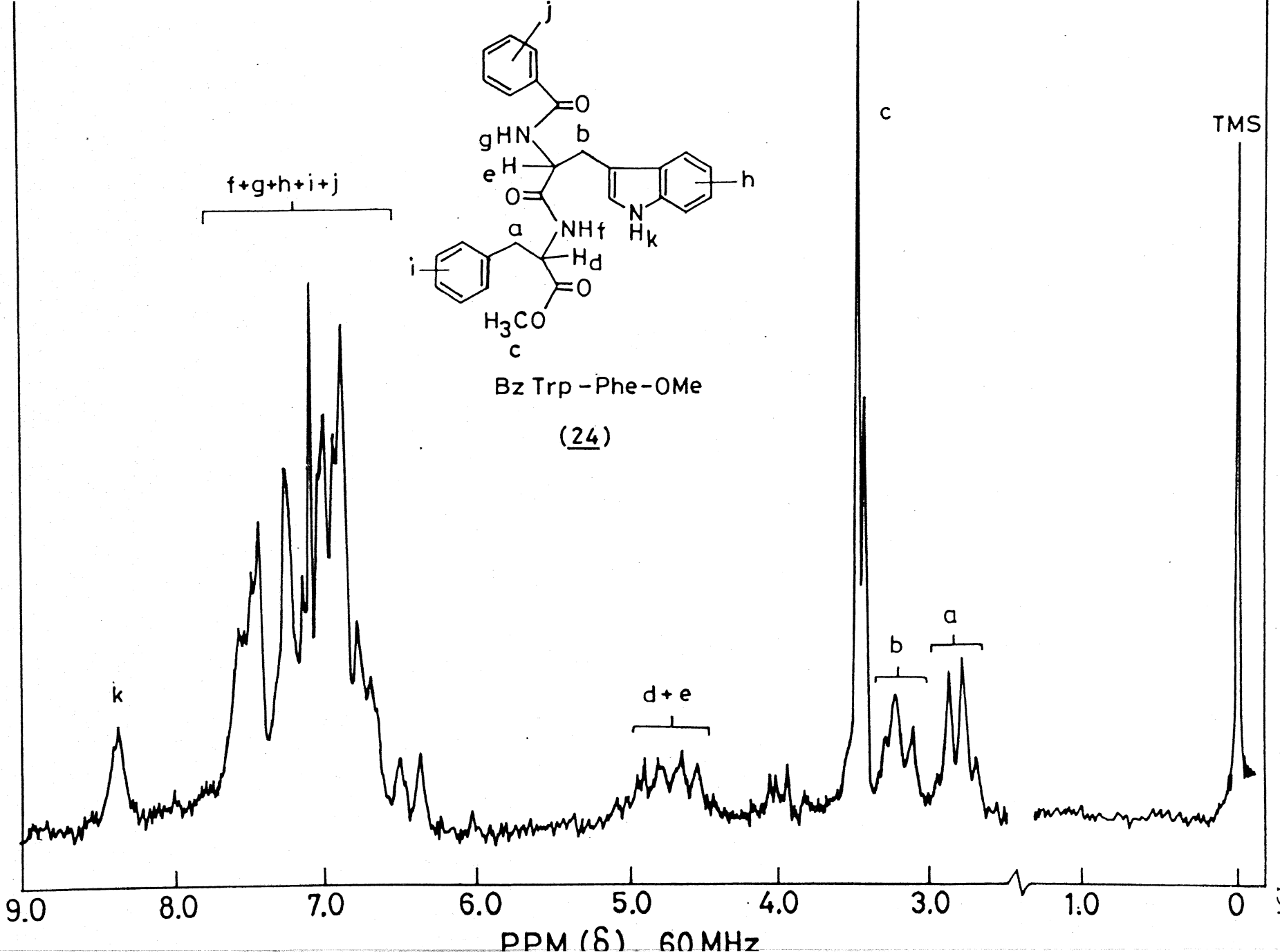

f
O
C
N
O
C-OCH3
He
d
c
b
a
Bz Pro OMe
(11)
f
CHCl3
e
c + d
a + b
TMS
9.0
8.0
7.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ), 60 MHz


f
O
H
N
O
d
H_3C-C-
$C-OCH_3$
a
H_e
b
$C-OCH_3$
O
c
Ac Asp (β - OMe) OMe
(18)
d
c
a
TMS
b
f
e
Sb
Sb
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ), 60 MHz



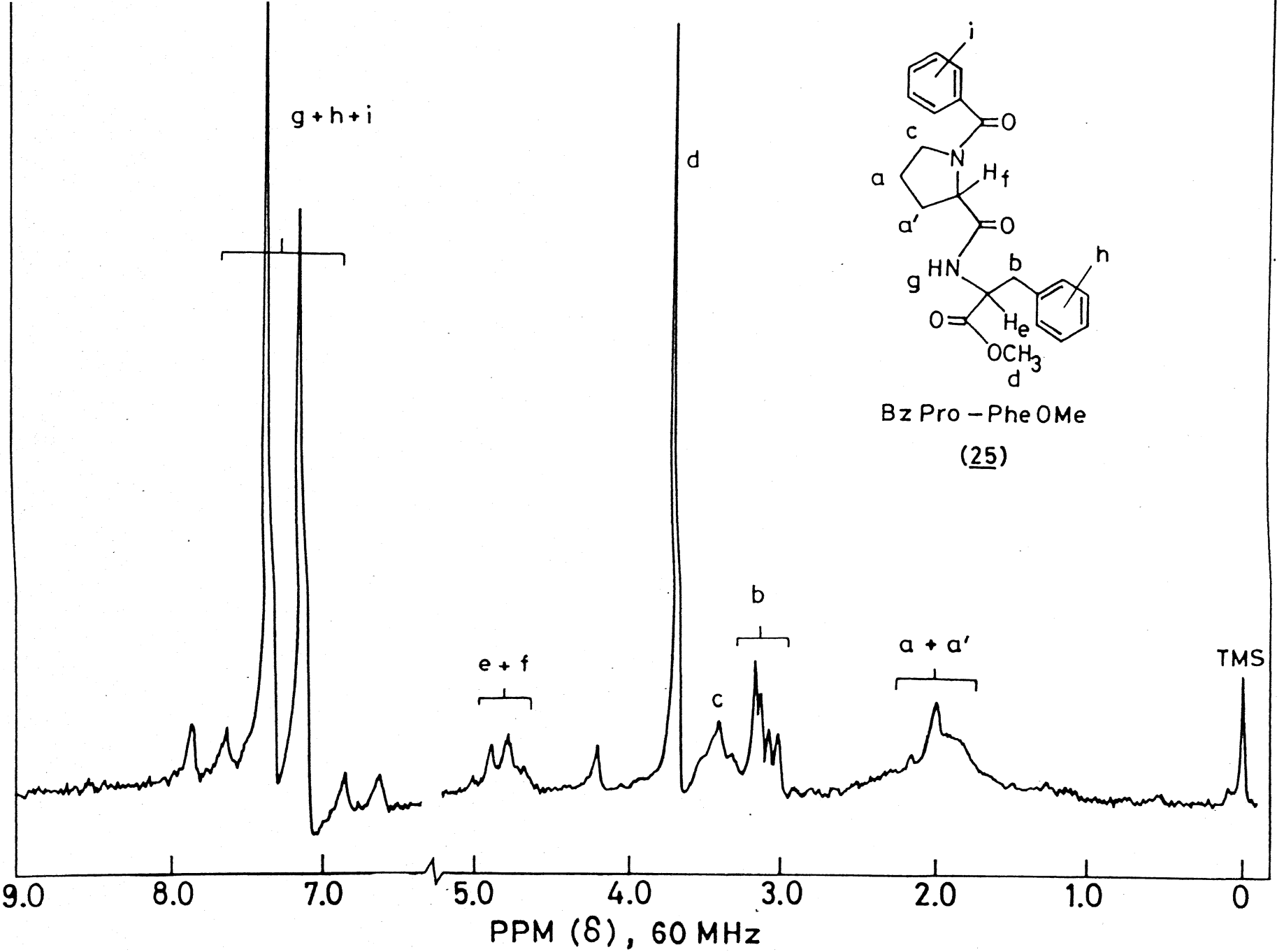
Bz Glu (Y-OMe) OMe

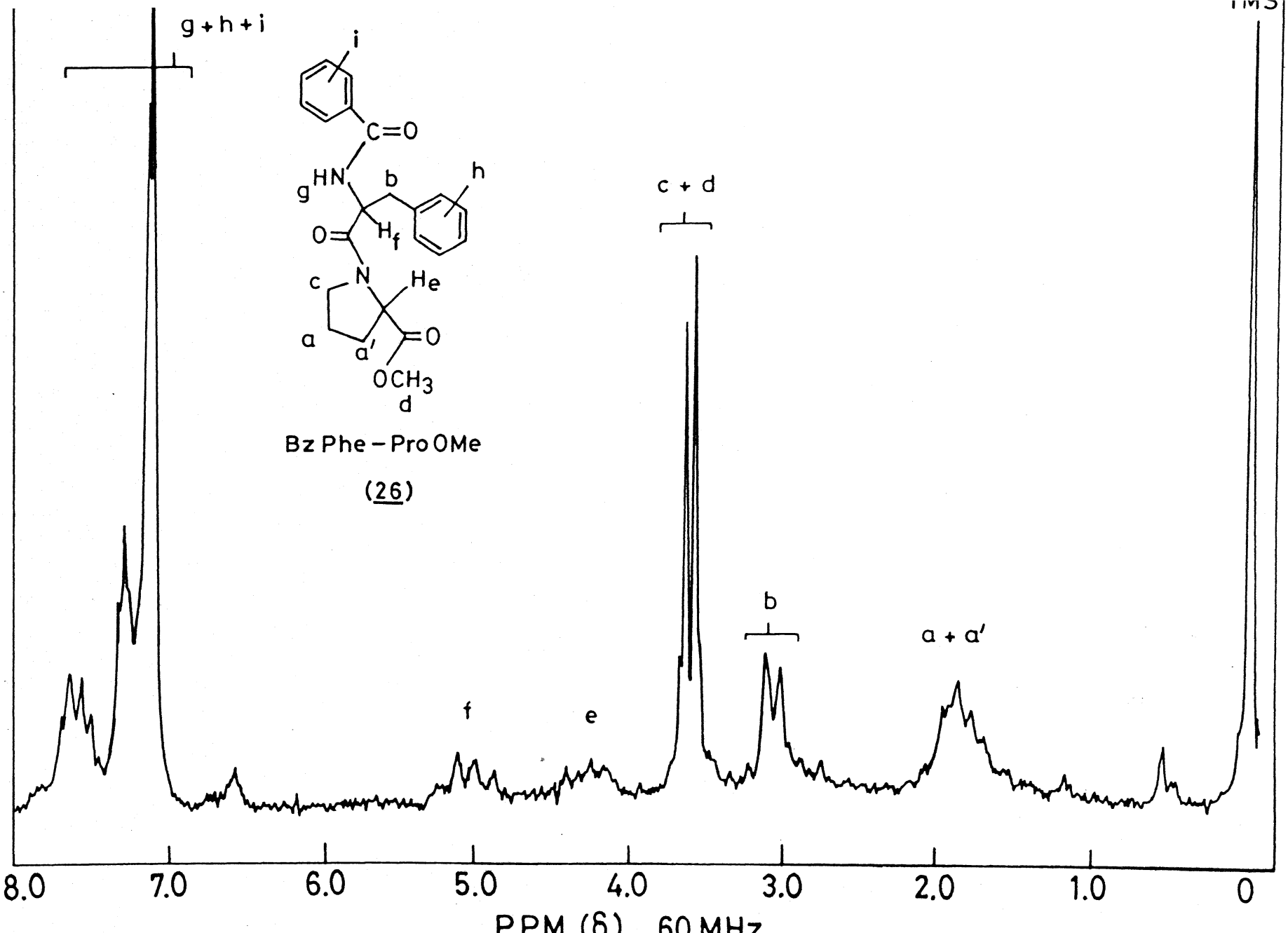
(20)

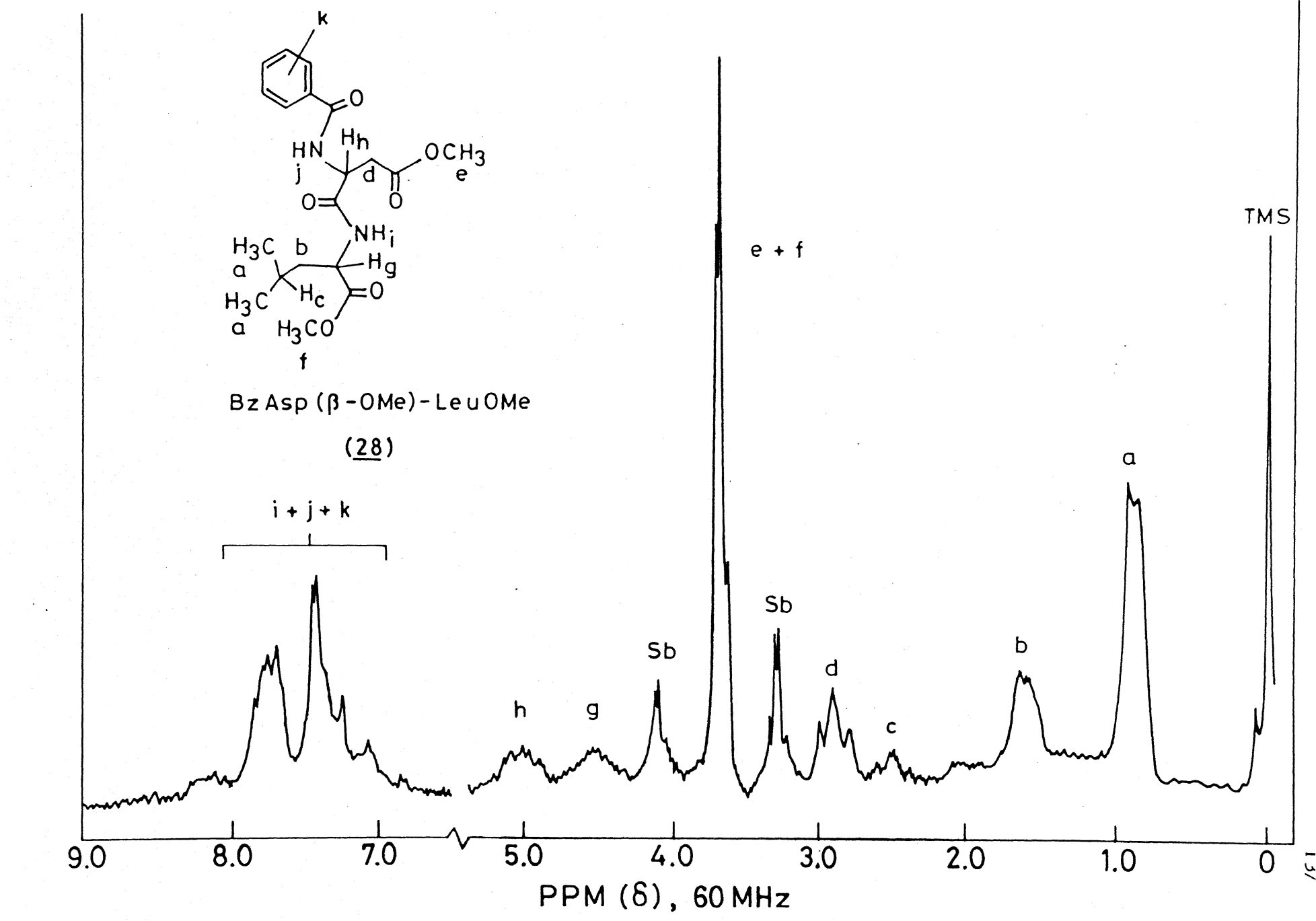


Bz Trp – Phe – OMe

(24)

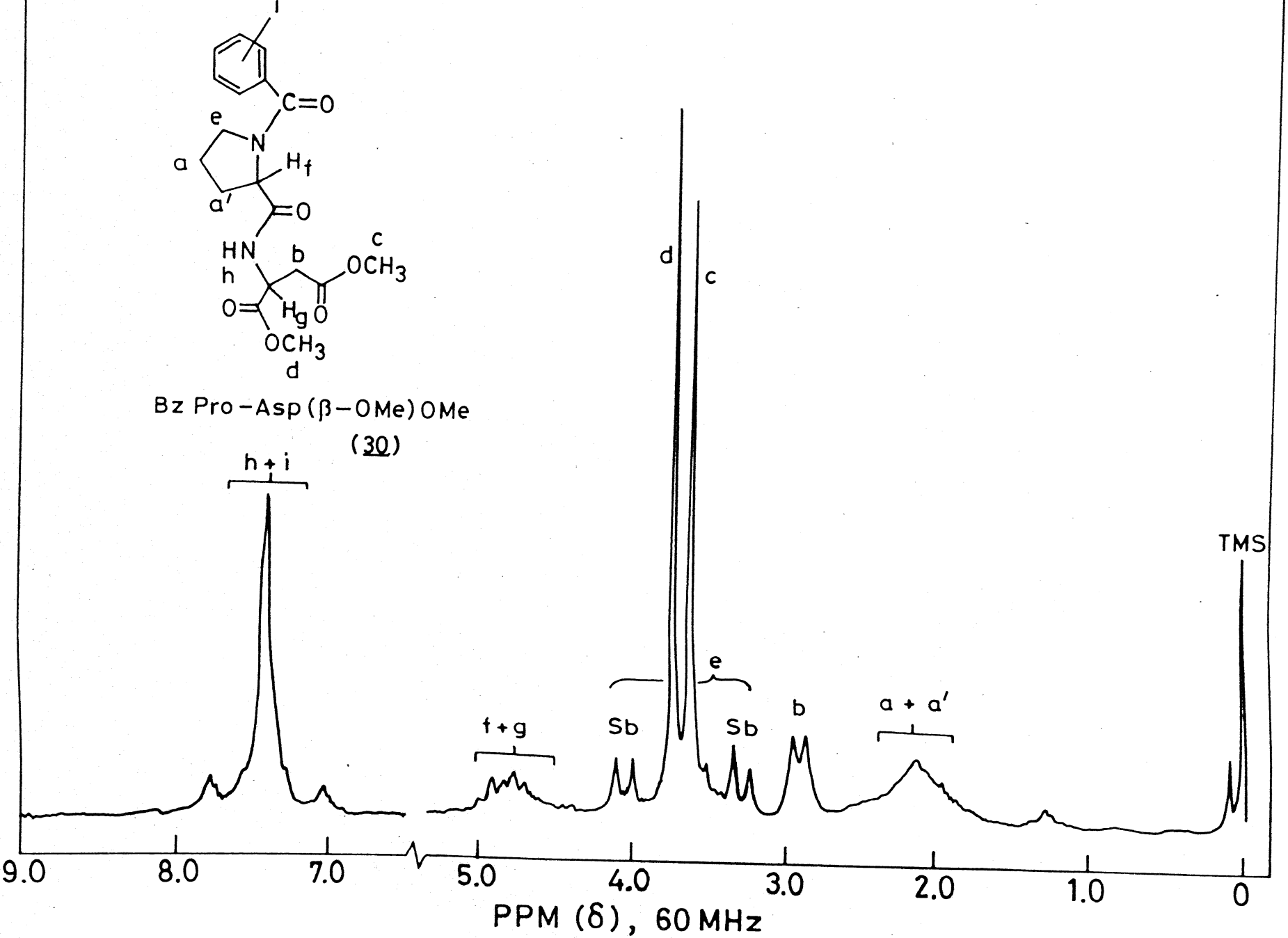

g + h + i
d
b
e + f
c
a + a'
TMS
9.0
8.0
7.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ), 60 MHz
i
c
N
O
a
H_f
a'
HN
g
b
h
H_e
OCH_3
d
Bz Pro – Phe OMe
(25)

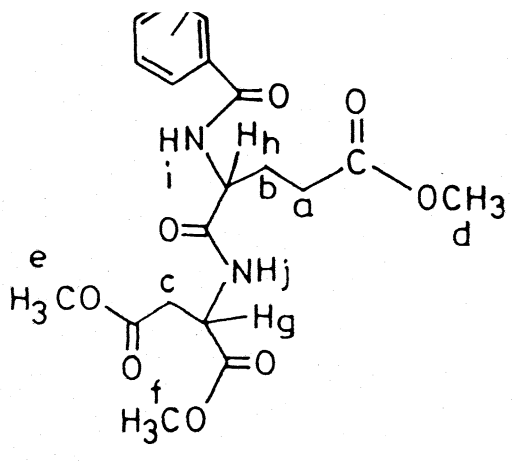

g + h + i
i
C=O
g HN
b
h
O
H_f
N
c
He
a
a'
O
OCH_3
d
Bz Phe – Pro OMe
(26)
c + d
b
a + a'
f
e
TMS
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ), 60 MHz


k
O
HN
Hh
j
d
OCH3
e
O
O
NHi
H3C
a
b
Hg
H3C
a
Hc
O
H3CO
f
Bz Asp (β-OMe)-Leu OMe
(28)
i + j + k
h
g
Sb
e + f
Sb
d
c
b
a
TMS
9.0
8.0
7.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ), 60 MHz

Bz Asp (β-OMe)-Phe OMe

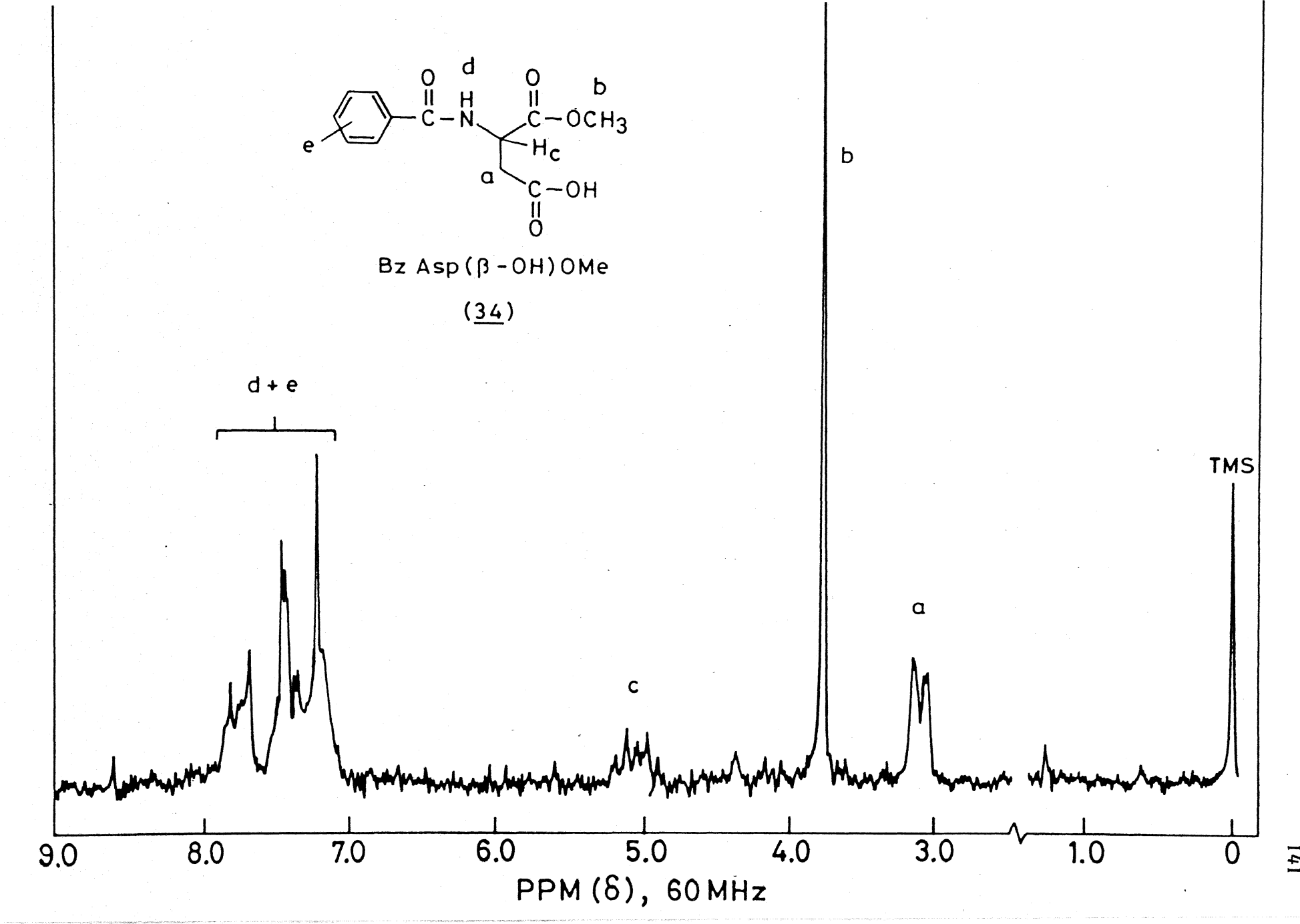
(29)

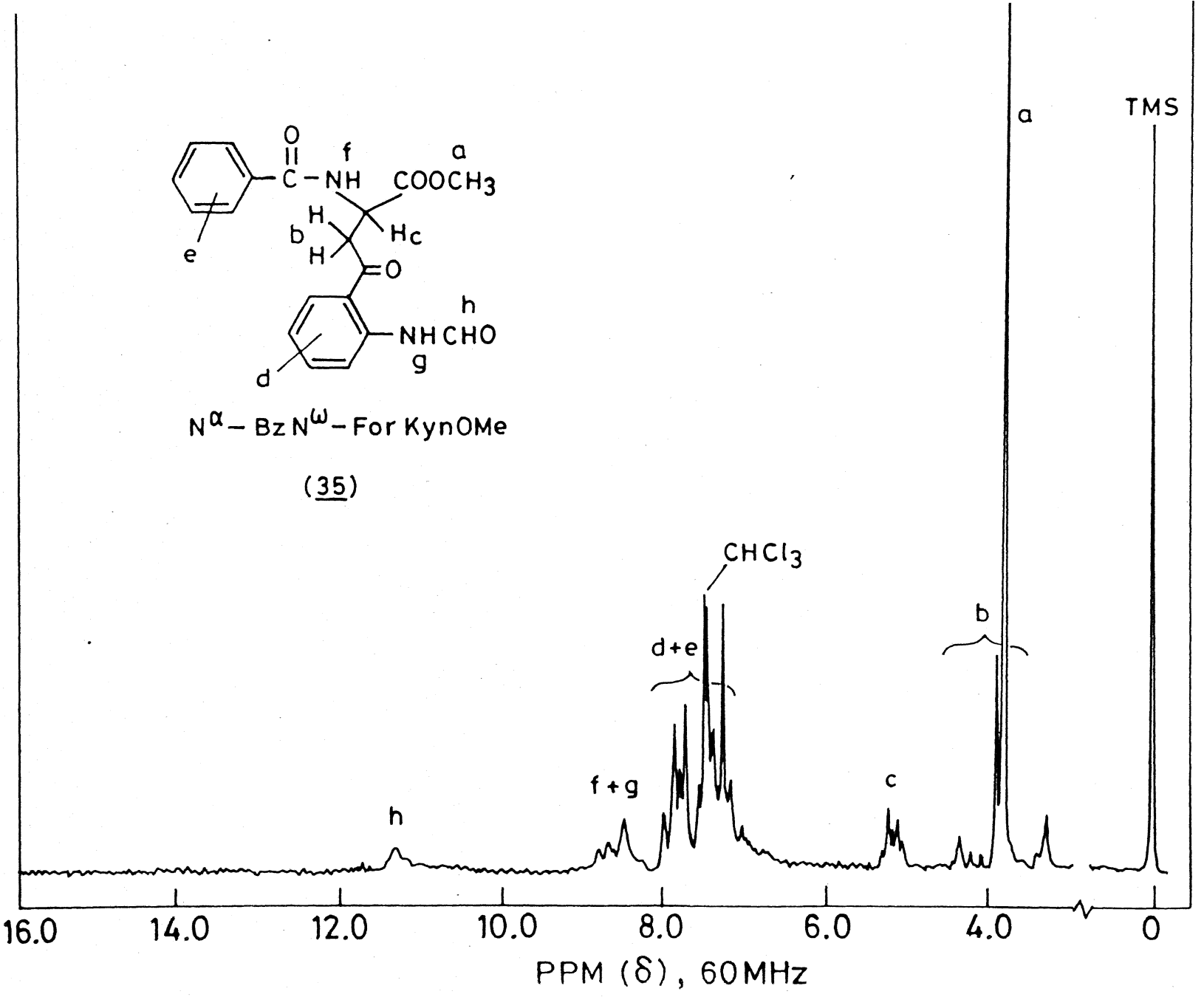

C=O
e
N
a
H_f
a'
O
HN
b
c
OCH_3
h
O
H_g
O
OCH_3
d
Bz Pro-Asp (β-OMe) OMe
(30)
h + i
f + g
Sb
d
c
e
Sb
b
a + a'
TMS
9.0
8.0
7.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ), 60 MHz



Bz Glu (γ-OMe)–Asp (β-OMe) OMe

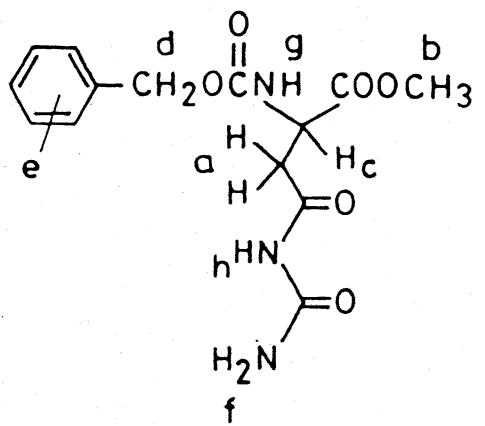
(31)


d
O
H
O
b
C–N
C–OCH3
e
Hc
a
C–OH
O
Bz Asp(β-OH)OMe
(34)
b
d + e
TMS
a
c
9.0
8.0
7.0
6.0
5.0
4.0
3.0
1.0
0
PPM (δ), 60 MHz



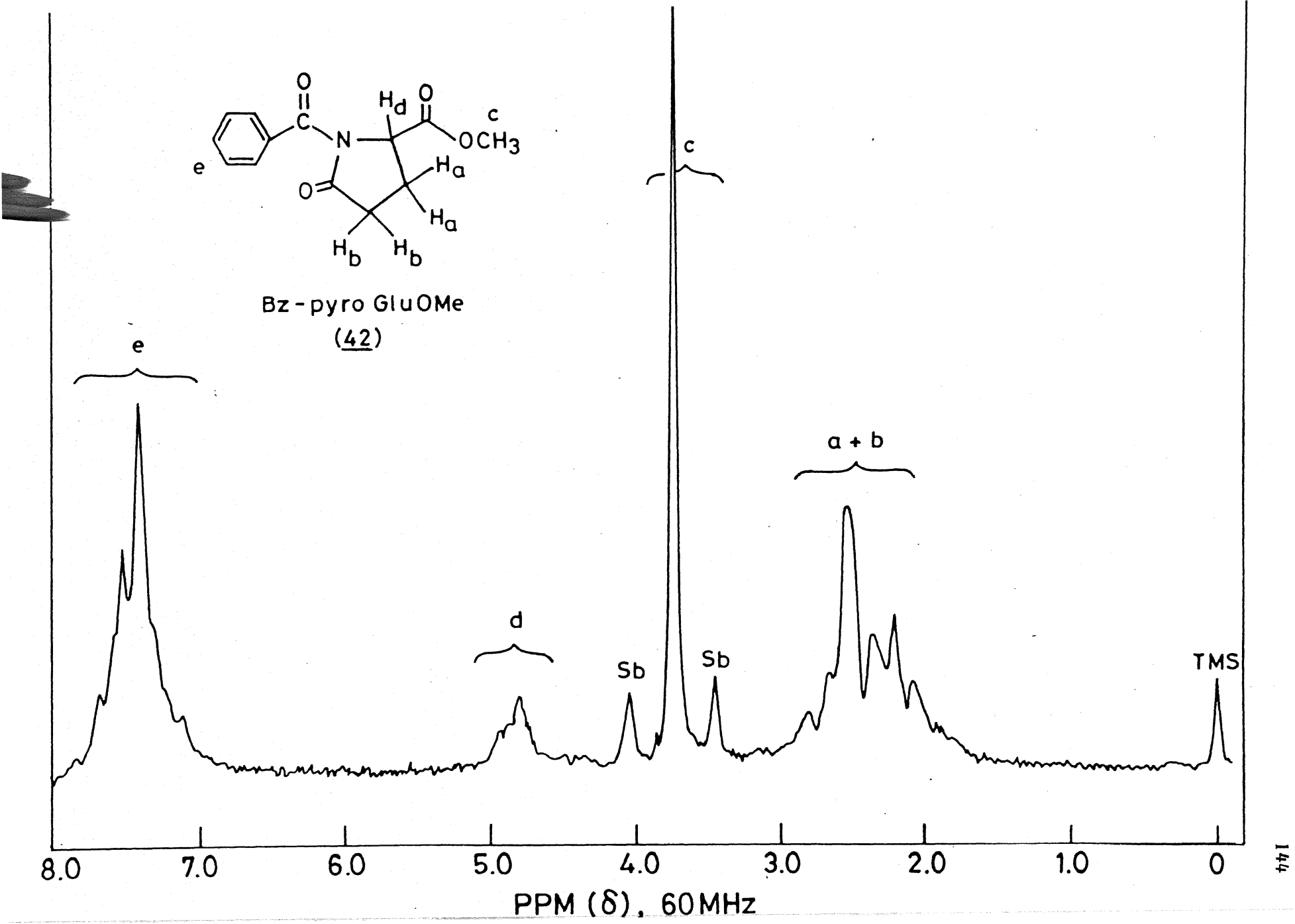
N^{α} – Bz N^{ω} – For KynOMe

(35)

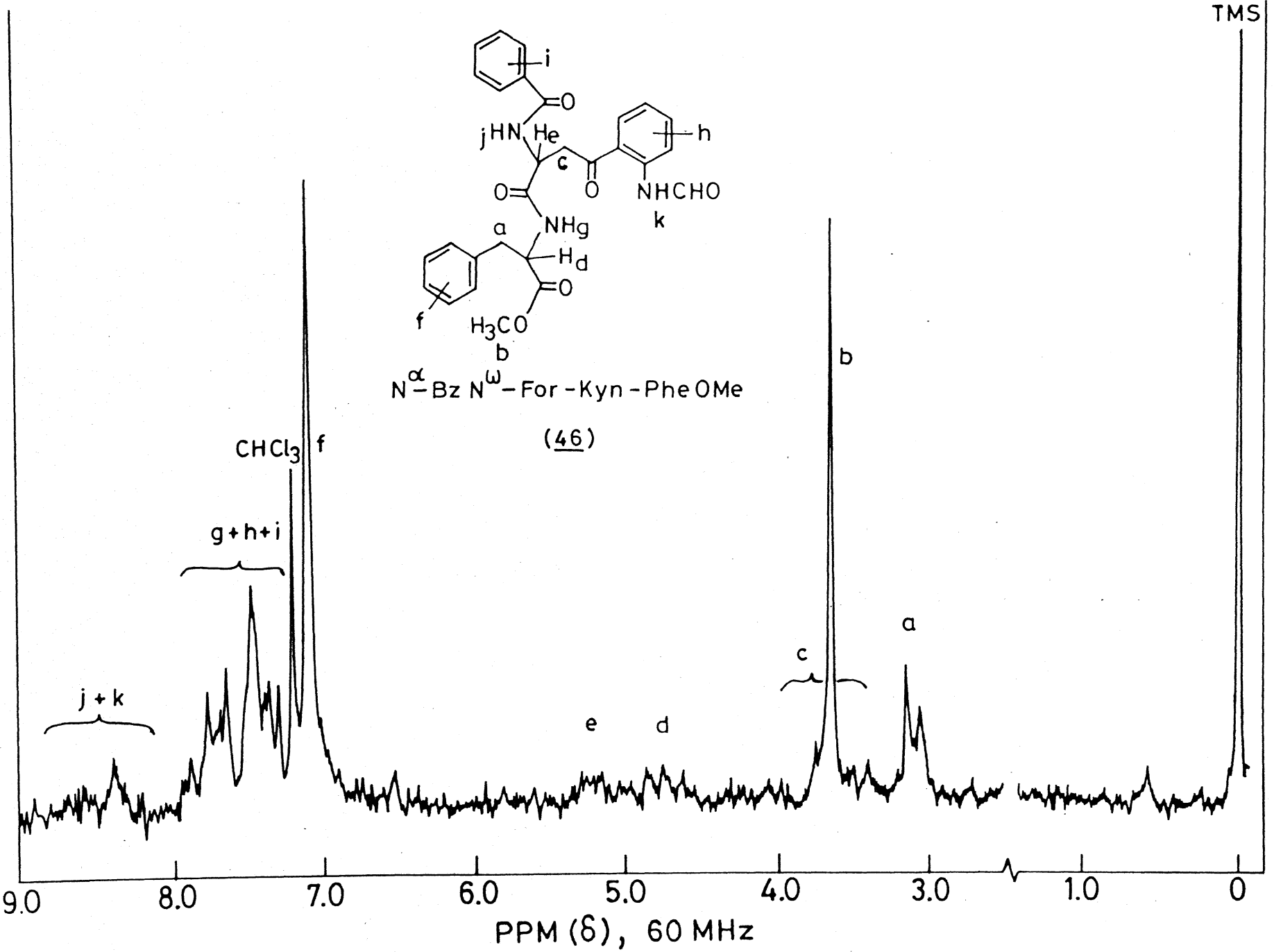


ZAsp(β-NHCONH$_2$)OMe

(36)

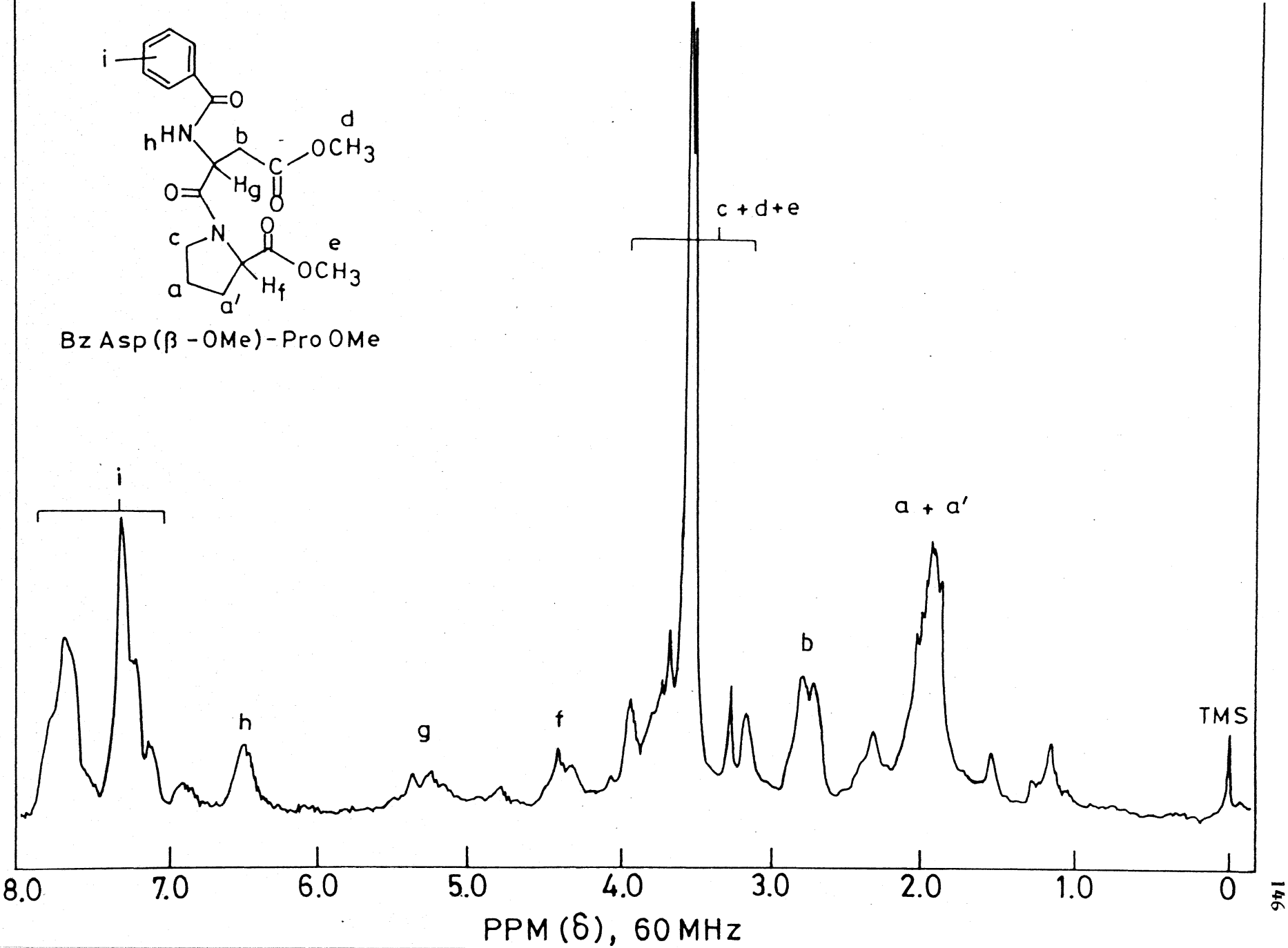


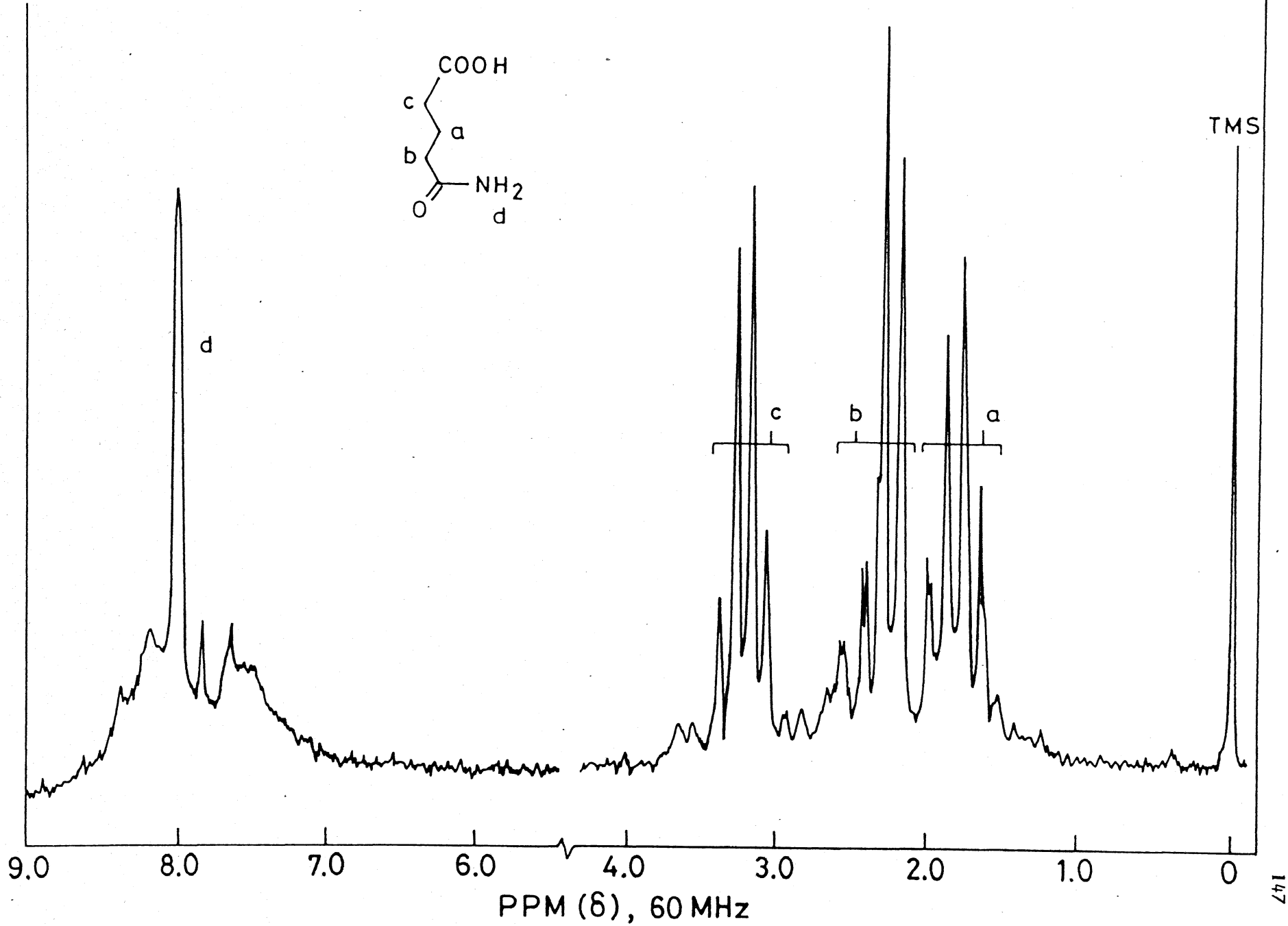
Bz-pyro GluOMe
(42)

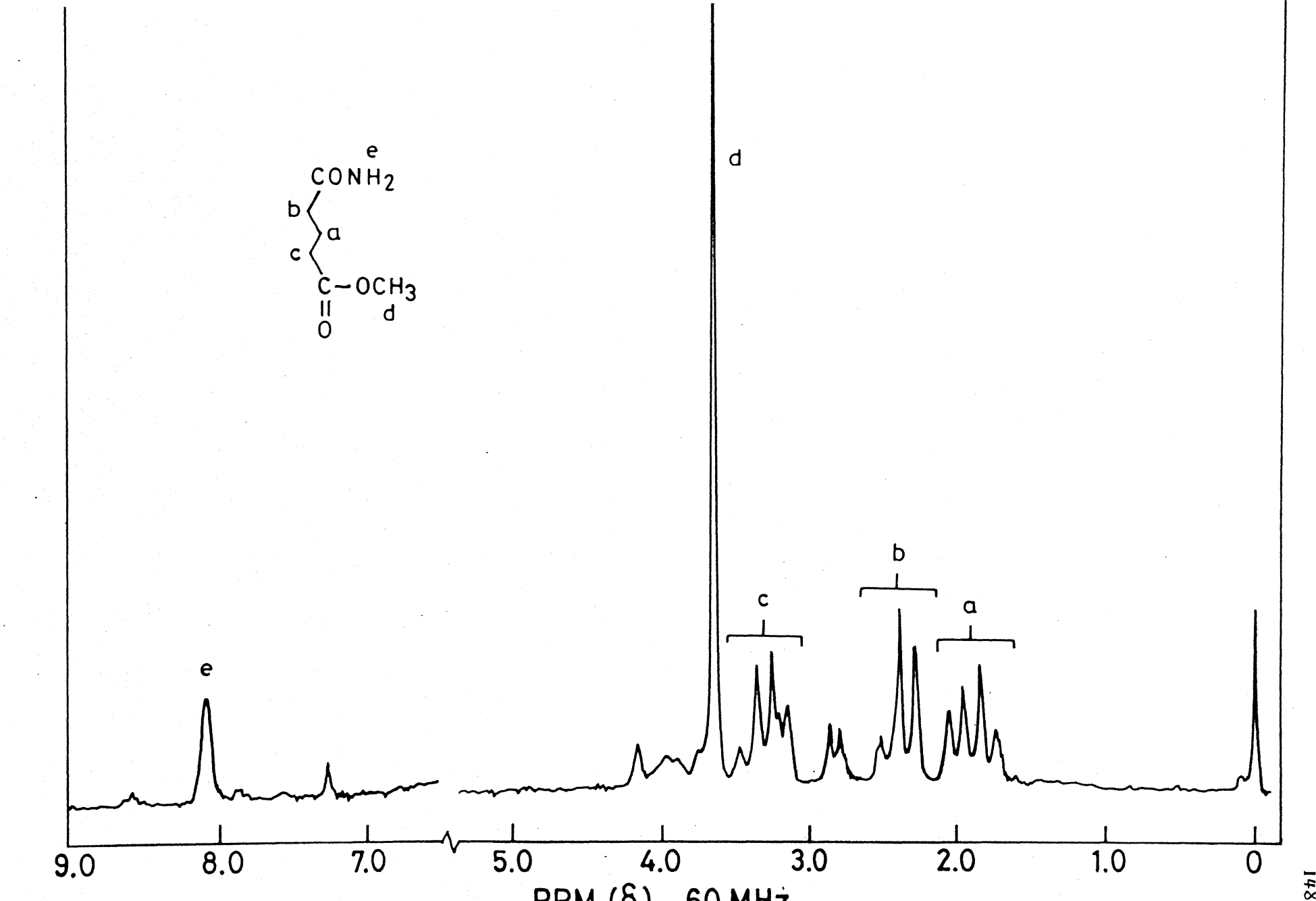


N^{α}-Bz N^{ω}-For-Kyn-Phe OMe

(46)


i
h HN
b
d
OCH3
C
Hg
O
O
O
c
N
e
OCH3
Hf
a
a'
Bz Asp (β -OMe)- Pro OMe
c + d + e
i
a + a'
b
h
g
f
TMS
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ), 60 MHz


COOH
c
a
b
NH2
O
d
TMS
d
c
b
a
9.0
8.0
7.0
6.0
4.0
3.0
2.0
1.0
0
PPM (δ), 60 MHz


e
CONH2
b
a
c
C–OCH3
O
d
d
e
c
b
a
9.0
8.0
7.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ), 60 MHz

SECTION E : EXPERIMENTAL

Melting points and boiling points are uncorrected. Infra-red spectra were recorded on Perkin - Elmer Model 580 or 377 spectrophotometers, either as neat liquids, or as thin KBr wafers. NMR spectra were obtained on dilute solutions in $CDCl_3$ or DMSO - D_6 on a Hitachi R600 (FT) spectrometer. In a few cases, spectra were recorded on 100 and 400 MHz instruments. The chemical shifts are reported in ppm with TMS at 0.00 as the internal standard. Mass spectra were recorded on a Jeol instrument. Elemental analyses were carried out in automatic C,H,N analysers. Silica gel G (ACME) was used for tlc and column chromatography (100-200 mesh). Reactions were monitored wherever possible by tlc. The organic extracts were invariably dried over anhydrous $MgSO_4$ and solvents evaporated in vacuo.

I. N-Benzyloxycarbonyl L-Phenylalanine Methyl Ester (ZPheOMe, 1) :

(i) Phenylalanine methyl ester hydrochloride (PheOMe.HCl) :

To stirred and ice-cooled dry MeOH (43.5 ml) was added, in drops, $SOCl_2$ (4.9 ml, 67 mmol) followed by L-Phe (9g, 54.5 mmol). The reaction mixture was allowed to attain rt, refluxed for 2 h, the clear solution evaporated, and the residue, on crystallization from dry MeOH-Et_2O, gave 10.5g (89%) of PheOMe. HCl, mp. 161°C (lit.[69] mp. 160°C).

ir : ν_{max}(KBr) cm^{-1} : 2830, 1740 (ester).

(ii) ZPheOMe (1) :

To an ice-cooled and stirred solution of PheOMe.HCl (1.079g, 5 mmol) in satd. aq. $NaHCO_3$ (~ 100 ml, pH ~ 9) was added, in drops, benzyloxycarbonyl chloride (Z-Cl) (2.5 ml, 50% in toluence, 5.698 mmol). The pH was maintained between 8-9 throughout the reaction. The mixture was left stirred for 4 h at rt, extracted with CH_2Cl_2 (3 x 50 ml), dried and evaporated to give 1.412 g (90%) of (1) as an oil.

ir : ν_{max}(neat) cm^{-1} : 3380 (br), 1735 (ester), 1620, 1525 (amide).

nmr : δ ($CDCl_3$) : 3.1 (d, 2H, -C<u>H</u>$_2$Ph), 3.65 (s, 3H, -COOC<u>H</u>$_3$), 4.6 (m, 1H, C<u>H</u>), 5.05 (s, 2H, -OC<u>H</u>$_2$Ph), 6.75 - 7.85 (m, 11H, -N<u>H</u>, aromatic).

I. N-Acetyl L-Phenylalanine Methyl Ester (AcPheOMe, 2) :

i) N-Acetyl phenylalanine (AcPhe-OH) :

To an ice-cooled and stirred solution of L-Phe (4.5g, 77.24 mmol) in N NaOH (15 ml) was added, in batches, acetic anhydride (7.7 ml, 81.72 mmol)

nd 2N NaOH (9 ml), alternately. The reaction mixture was allowed to attain , adjusted to pH 3 with 6N H_2SO_4, saturated with NaCl and extracted with tOAc (3 x 30 ml). The organic extract was washed with water, dried and evapo- ted to yield AcPhe-OH, mp. 167-169°C; yield 4.5g (80%) (lit.[70] mp. 171-172°C).

ir : ν_{max}(KBr) cm^{-1} : 3360, 1710 (acid), 1630, 1560 (amide).

) AcPheOMe (2) :

To stirred and ice-cooled dry MeOH (8.7 ml), $SOCl_2$ (1 ml, 13.4 mmol), drops, followed by AcPhe-OH (2.26 g, 11 mmol) was added, the reaction mix- re allowed to attain rt, refluxed for 2 h, solvents evaporated and the residue chromatography over silica gel and elution with PhH:EtOAc :: 7:3 gave AcPheOMe,),(1.83 g, 75%), mp. 80°C (PhH-Hexane).

ir : ν_{max}(KBr) cm^{-1} : 3370, 1765 (ester), 1660, 1550 (amide).

nmr : $\delta(CDCl_3)$: 1.95 (s, 3H, $-COC\underline{H}_3$), 3.1 (d, 2H, $-C\underline{H}_2Ph$), 3.7 (s, 3H, $-COOC\underline{H}_3$), 4.85 (q, 1H, $-C\underline{H}$), 5.95 (br, 1H, $-N\underline{H}$), 6.9 - 7.4 (m, 5H, aromatic protons).

. N-Benzyloxycarbonyl Glycine Methyl Ester (ZGlyOMe, 3) :

Glycine methyl ester hydrochloride (GlyOMe.HCl) :

To a stirred suspension of glycine (20g, 0.26 mol) in dry MeOH !5 ml), dry HCl was passed at rt until a clear solution was obtained. The reaction xture was cooled (0 - 5°C) and the passage of dry HCl continued till saturation. e solvents were evaporated in vacuo and the residue on crystallization from / MeOH-Et_2O gave GlyOMe.HCl, 28 g (86%), mp. 175-176°C (lit.[71] mp. 175°C).

ir : ν_{max}(KBr) cm^{-1} : 2960, 1740 (ester).

(ii) ZGlyOMe (3) :

The reaction of GlyOMe.HCl (1.26 g, 10 mmol) with Z-Cl (2.8 ml, 50% in toluene, 12 mmol), precisely as described in Experiment-I, gave, ZGlyOMe, (3), 2.05 g (92%) as an oil ; bp. 168 - 170°C/ 1 torr.

ir : ν_{max}(neat) cm^{-1} : 3350, 1720 (ester), 1520.

nmr : $\delta(CDCl_3)$: 3.7 (s, 3H, $-COOC\underline{H}_3$), 3.9 (d, 2H, $-C\underline{H}_2COOCH_3$), 5.1 (s, 2H, $-OC\underline{H}_2Ph$), 7.25 (s, 6H, -NH, aromatic protons).

IV. N-Benzoyl L-Tyrosine Methyl Ester (BzTyrOMe, 4) :

(i) Tyrosine methyl ester hydrochloride (TyrOMe.HCl) :

To stirred and ice-cooled dry MeOH (35 ml) was added, in drops, $SOCl_2$(4 ml, 58 mmol) followed by L-Tyr (9 g, 50 mmol). The reaction mixture was allowed to attain rt, left aside for 2 h under stirring, refluxed for an additional 2 h, solvents evaporated and the residue on crystallization from dry MeOH-Et_2O gave 9.0 g (78%) of TyrOMe.HCl ; mp. 188°C (lit.[72] mp. 190°C).

ir : ν_{max}(KBr) cm^{-1} : 3330, 2880, 1730 (ester).

(ii) BzTyrOMe (4) :

Benzoyl chloride (1.5 ml, 12.8 mmol) was added to a vigorously shaken mixture of TyrOMe.HCl (3 g, 16 mmol), 2N $NaHCO_3$ (26 ml), $CHCl_3$ (130 ml) and H_2O (6.5 ml) contained in a separatory funnel. The addition was carried out in four lots. The organic layer was separated, dried, evaporated and the residue on crystallization from hexane followed by washing the product with

EtOAc : Hexane :: 1:1 gave 3.2 g (83%) of BzTyrOMe, mp. 156°C.

ir : ν_{max}(KBr) cm^{-1} : 3260, 1695 (ester), 1635, 1525 (amide).

nmr : $\delta(CDCl_3 + DMSO\text{-}d_6)$: 3.1 (d, 2H, -CHC<u>H</u>$_2$), 3.7 (s, 3H, -COOC<u>H</u>$_3$), 4.85 (q, 1H, -C<u>H</u>), 6.55 - 7.95 (m, 10H, -N<u>H</u>, aromatic protons), 8.75 (s, 1H, -O<u>H</u>).

V. N-Benzyloxycarbonyl L-Tryptophan Methyl Ester (ZTrpOMe, <u>5</u>) :

(i) Tryptophan methyl ester hydrochloride (TrpOMe.HCl) :

To ice-salt cooled (-10°C) and stirred dry MeOH (25 ml) was added, in drops, $SOCl_2$ (1.9 ml, 23.6 mmol) followed by, rapidly, L-Trp (2 g, 12.5 mmol). From the resulting clear solution, after a short while, a solid precipitated. The reaction mixture was left stirred for an additional 4 h at -5 to 0°C, allowed to attain rt, when the precipitated solid redissolved. The resulting clear yellow solution was left stirred at rt overnight, concentrated in vacuo to ~ 5 ml, admixed with dry Et_2O, the mixture refrigerated for 4 h, filtered and dried to yield TrpOMe.HCl (3 g, 95%) ; mp. 214°C (MeOH-Et_2O) (lit.[73] mp. 213.5 - 214°C).

ir : ν_{max}(KBr) cm^{-1} : 3270, 1740 (ester).

(ii) ZTrpOMe (<u>5</u>) :

The reaction of TrpOMe.HCl (1 g, 3.93 mmol) and Z-Cl (2.5 ml, 50% in toluene, 5.7 mmol), precisely as described in Experiment-I, gave ZTrpOMe (<u>5</u>) (2.45 g, 70%) as a thick oil which could be further purified by bulb-to-bulb distillation ; bp. 230 - 240°C/ 0.5 torr.

VI. N-Benzoyl L-Tryptophan Methyl Ester (BzTrpOMe, 6) :

To an ice-salt cooled (-10°C) and stirred solution of TrpOMe.HCl (2.55 g, 10 mmol) in satd. aq. $NaHCO_3$ (~ 150 ml) was added, over 0.5 h, Bz-Cl(1.2ml, 10.24 mmol). The resulting gummy material was dissolved by addition of minimum amount of benzene and left stirred for 20 h at rt. The benzene layer was separated and admixed with EtOAc to obtain clarity. The aqueous layer was extracted with EtOAc (3 x 30 ml), the combined organic layers dried and evaporated to yield 2.65 g (82%) of BzTrpOMe,(6), mp. 110-111°C.

ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1720 (ester), 1620, 1575.

nmr : $\delta(CDCl_3)$: 3.4 (d, 2H, -CHC$\underline{H_2}$), 3.65 (s, 3H, -COOC$\underline{H_3}$), 5.1 (m, 1H, -C$\underline{H}$), 6.6 - 7.9 (m, 11H, -N$\underline{H}$, aromatic protons), 8.65 (br s, 1H, -N$\underline{H}$).

VII. N-Benzyloxycarbonyl L-Histidine Methyl Ester (ZHisOMe, 7) :

(i) Histidine methyl ester dihydrochloride (HisOMe.2HCl) :

A mixture of L-His.monoHCl (10 g, 52.14 mmol), dry MeOH (150 ml) and conc. H_2SO_4 (2.6 ml) was refluxed for 1 h , subjected to passage of dry HCl for 2 h, cooled and evaporated to yield HisOMe.2HCl (11.8 g, 94%) ; mp. 198°C (MeOH-Et_2O) (lit.[74] mp. 200 - 201°C).

ir : ν_{max}(KBr) cm^{-1} : 3100, 1750 (ester).

(ii) ZHisOMe (7) :

To an ice-cooled and stirred solution of HisOMe.2HCl (3.0 g, 12.4 mmol) in dry $CHCl_3$ (24.8 ml) admixed with dry Et_3N (3.46 ml, 25 mmol), was added Z-Cl (2.72 ml, 50% in toluene, 6.2 mmol), followed by, after 2 min, the second

batch of Et_3N (1.74 ml, 12.4 mmol) and Z-Cl (2.72 ml, 50% in toluene, 6.2 mmol). The mixture was left stirred at 0°C for 0.25 h, then at rt for 0.5 h, washed with water (3 x 10 ml), dried, evaporated, mixed with dry EtOH (3 x 15 ml) and evaporated. The EtOH addition and evaporation sequence was repeated thrice to yield 3.6 g (96%) of ZHisOMe, (7), as an oil.[75]

ir : ν_{max}(neat) cm^{-1} : 3200 (br), 1715 (ester), 1520, 1440.

nmr : $\delta(CDCl_3)$: 3.1 (d, 2H, -CHC$\underline{H}_2$), 3.65 (s, 3H, -COOC$\underline{H}_3$), 4.55 (br, 1H, -C$\underline{H}$), 5.05 (s, 2H, -OC$\underline{H}_2$Ph), 6.3 (br, 1H, -N$\underline{H}$), 6.72 (br, 1H, -NC$\underline{H}$=C), 7.3 (s, s, 5H, -$CH_2C_6\underline{H}_5$), 7.8 (br, 1H, -NHC$\underline{H}$=N-).

VIII. N-Benzoyl L-Methionine Methyl Ester (BzMetOMe, 8) :

(i) Methionine methyl ester hydrochloride (MetOMe.HCl) :

To an ice-cooled and stirred solution of L-Met (10.45 g, 70 mmol) in dry MeOH (70 ml) was added, in drops, $SOCl_2$ (7 ml, 86.9 mmol). The reaction mixture was allowed to attain rt, left stirred overnight, solvents evaporated in vacuo, the residue triturated with dry Et_2O and the resulting solid, on crystallization from dry MeOH - Et_2O gave 11.47 g (82%) of MetOMe.HCl, mp. 150°C (lit.[76] mp. 151°C).

ir : ν_{max}(KBr) cm^{-1} :: 3410, 1730 (ester).

(ii) BzMetOMe (8) :

Under vigorous shaking, Bz-Cl (10.54 g, 75 mmol) was added, during 0.5 h, to a solution of MetOMe.HCl (10 g, 50 mmol) in satd. aq. $NaHCO_3$ (250 ml) held at 0°C, keeping the medium basic throughout. The reaction mixture was left stirred for 12 h, extracted with Et_2O (3 x 30 ml), dried and evaporated to yield 12 g (90%) of BzMetOMe, (8), mp. 75°C (dry PhH-Hexane).

ir : ν_{max}(KBr) cm^{-1} : 3290 (-NH), 1740 (ester), 1630, 1525 (amide), 1420.

nmr : $\delta(CDCl_3)$: 2.1 (s, 3H, -SC<u>H</u>$_3$), 2.2 - 2.8 (m, 4H, -CH(C<u>H</u>$_2$)$_2$), 3.75 (s, 3H, -COOC<u>H</u>$_3$), 4.9 (q, 1H, -C<u>H</u>), 6.9 - 8.0 (m, 6H, -N<u>H</u>, aromatic).

IX. N-Benzyloxycarbonyl L-Methionine Methyl Ester (ZMetOMe, <u>9</u>) :

MetOMe.HCl (2 g, 10 mmol) was dissolved in 2.7 ml of water and 27 ml of EtOAc was added to it. The free ester was formed at 0°C by the addition of an excess of satd. aq. $KHCO_3$ and Z-Cl (2.5 ml, 95% in toluene, 10.8 mmol) was then added to it. The reaction mixture was left stirred overnight at rt, the excess Z-Cl destroyed with pyridine, the EtOAc layer washed successively with water, dil. HCl and water and dried to yield ZMetOMe, (<u>9</u>), (2.66 g, 90%) as an oil.[76]

ir : ν_{max}(neat) cm^{-1} : 3315 (-NH), 1715 (ester), 1520, 1430.

nmr : $\delta(CDCl_3)$: 2.05 (s, 3H, -SC<u>H</u>$_3$), 2.15 - 2.75 (m, 4H, -CH(C<u>H</u>$_2$)$_2$), 3.7 (s, 3H, -COOC<u>H</u>$_3$), 4.35 - 4.75 (m, 1H, -C<u>H</u>), 5.05 (s, 2H, -OC<u>H</u>$_2$Ph), 5.5 (br, 1H, -N<u>H</u>), 7.25 (s, 5H, aromatic protons).

X. N-Benzyloxycarbonyl S-Benzyl L-Cysteine Methyl Ester (Z-Cys(S-Bzl)OMe, <u>10</u>) :

To an ice-cooled and stirred solution of Cys(S-Bzl)OMe.HCl (1.5 g, 5.74 mmol) in satd. aq. $NaHCO_3$ (100 ml) was added, in small portions, Z-Cl (2.0 ml, 95%, 8.7 mmol). Work-up, precisely as described in Experiment - I, gave Z-Cys(S-Bzl)-OMe, (<u>10</u>), (1.92 g, 93%), mp. 64 - 65°C (Hexane) (lit.[77] mp. 66 - 67°C).

ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1735 (ester), 1690, 1535.

nmr : $\delta(CDCl_3)$: 2.9 (d, 2H, -CHC<u>H</u>$_2$), 3.7 (s, s, 5H, -COOC<u>H</u>$_3$, -SC<u>H</u>$_2$Ph),

4.65 (br, 1H, -C<u>H</u>), 5.1 (s, 2H, -OC<u>H</u>$_2$Ph), 5.35 - 5.7 (br, 1H, -N<u>H</u>), 7.25, 7.35 (s, s, 10H, aromatic protons).

XI. N-Benzoyl L-Proline Methyl Ester (BzProOMe, <u>11</u>) :

(i) Proline methyl ester hydrochloride (ProOMe.HCl) :

To an ice-salt cooled solution of L-Pro (11.5 g, 0.1 mol) in dry MeOH (125 ml), gaseous HCl was passed till saturation and solvents removed in vacuo. The resulting residue was redissolved in dry MeOH, cooled, saturated with dry HCl and evaporated. The resulting oil was thoroughly dried over NaOH in vacuo to give 10.5 g (64%) of ProOMe.HCl as an oil.[78] This was used as such for the next reaction.

(ii) BzProOMe (<u>11</u>) :

Benzoylation of ProOMe.HCl (1 g, 6.04 mmol) with Bz-Cl (1.17 g, 8 mmol) in satd. aq. $NaHCO_3$ (70 ml) and work-up, precisely as described in Experiment - VIII, gave 1.3 g (93%) of BzProOMe,(<u>11</u>), mp. 89°C (dry PhH-Hexane).

ir : ν_{max}(KBr) cm^{-1} : 1735 (ester), 1615, 1570, 1410.

nmr : $\delta(CDCl_3)$: 1.5 - 2.6 (m, 4H, -CH(C<u>H</u>$_2$)$_2$), 3.65 (m, 5H, -COOC<u>H</u>$_3$, -NC<u>H</u>$_2$), 4.45 - 4.85 (br, 1H, -C<u>H</u>), 6.9 - 8.0 (m, 5H, aromatic).

XII. N-Benzoyl L-Serine Methyl Ester (BzSerOMe, <u>12</u>) :

(i) Serine methyl ester hydrochloride (SerOMe.HCl) :

To a stirred suspension of L-Ser (10.5 g, 0.1 mol) in dry MeOH (80 ml) was passed dry HCl until saturation when a clear solution was obtained. Solvents

were evaporated in vacuo, the residue redissolved in dry MeOH, saturated with dry HCl, evaporated, the residue crystallized from dry MeOH-Et_2O, filtered, washed with Et_2O and dried in vacuo over KOH to give 13.9 g (90%) of SerOMe.HCl, mp. 166°C (lit.[79] mp. 166°C).

ir : ν_{max}(KBr) cm^{-1} : 3380, 1730 (ester).

(ii) BzSerOMe (12) :

To an ice-cooled and vigorously shaken solution of SerOMe.HCl (6 g, 38.4 mmol) in satd. aq. $NaHCO_3$ (300 ml) was added, in drops, Bz-Cl (4.5 ml, 38 mmol). The reaction mixture was kept stirred for 3 h maintaining the medium basic throughout, extracted with Et_2O (3 x 100 ml), dried, evaporated, and the residue on crystallization from dry PhH-Hexane, gave, 7.12 g (83%) of BzSerOMe, mp. 86°C (lit.[51] mp. 86°C).

ir : ν_{max}(KBr) cm^{-1} : 3430 (-OH), 3300 (-NH), 1740 (ester), 1620, 1530 (amide).

nmr : $\delta(CDCl_3)$: 3.15 (br, 1H, $-CH_2\underline{OH}$), 3.7 (s, 3H, $-COOC\underline{H_3}$), 3.95 (d, 2H, $-CHC\underline{H_2}$), 4.8 (m, 1H, $-C\underline{H}$), 7.1 - 8.05 (m, 6H, $-N\underline{H}$, aromatic).

XIII. N-Benzoyl L-Threonine Methyl Ester (BzThrOMe, 13) :

(i) Benzoyl threonine (BzThr-OH) :

Under vigorous shaking and ice-salt cooling, Bz-Cl (5.4 ml, 46.2 mmol) was added, in portions, to a solution of L-Thr (5.0 g, 42 mmol) in 1 N NaOH (100 ml). The reaction mixture was left stirred overnight, made acidic to pH~ 2 (2N HCl), refrigerated for 2 h, filtered, washed with ice-cold water, then with CCl_4(3 x 15 ml) and dried to give 5.02 g (54%) of BzThr-OH, mp. 134-135°C (lit.[80] mp. 143-144°C).

(ii) BzThrOMe (13) :

To a solution of BzThrOH (1 g, 4.5 mmol) in MeOH (20 ml) was added an excess of a ice-cold ethereal solution of CH_2N_2. The reaction mixture was evaporated, and the residue on crystallization from EtOAc-Hexane gave 0.84 g (79%) of BzThrOMe, mp. 91-92°C (lit.[81] mp. 96°C).

ir : ν_{max}(KBr) cm^{-1} : 3410 (-OH), 3345 (-NH), 1730 (ester), 1630, 1510 (amide).

XIV. N^{α}-Benzyloxycarbonyl L-Lysine (N^{α}-Z Lys-OH, 14) :

(i) N^{ω}-Benzylidene L-Lysine :

Under vigorous shaking and ice-salt cooling, cold, freshly distilled PhCHO (6.36 g, 60 mmol) was added to a solution of L-Lys.HCl (9.1 g, 50 mmol) in 2N NaOH (42 ml). The shaking was continued for an additional 5 min. when the product separated out which was filtered, washed with cold water, Et_2O and dried to give 5.72 g (47%) of N^{ω}-Benzylidene L-lysine, mp. 203-205°C (lit.[82] mp. 205 - 207°C).

(ii) N^{α}-Z Lys-OH (14) :

To a vigorously shaken ice-cooled solution of N^{ω}-benzylidene lysine (5.72 g, 23.4 mmol) in 1N NaOH (24.4 ml) was added, alternately, Z-Cl (8.2 ml, 50% in toluene, 18.69 mmol) and 1N NaOH (36.6 ml), maintaining the medium basic throughout. The reaction mixture was left stirred for 0.25 h, adjusted to pH~2 with conc. HCl (6.1 ml), held at 50°C for 10 min, cooled and extracted with Et_2O (2 x 50 ml). The aqueous layer was adjusted to pH 6.2 with aq. NH_3, evaporated, the residue triturated with hot MeOH and the MeOH

extract on evaporation gave residue, mp. 215-216°C which was triturated with EtOAc. The EtOAc insoluble portion was digested with cold MeOH to yield residue-A and filterate. The latter was admixed with EtOAc until turbid, left refrigerated overnight and filtered to yield residue, mp. 200°C, which, on further crystallization from MeOH gave product, (14), mp. 232-233°C (D), (0.420 g). Residue-A was extracted with hot MeOH (3 ml), the MeOH extract admixed with EtOAc until turbid, left at rt overnight and filtered to give product, mp. 250-270°C (D). The combined filterates from the previous composition were evaporated and crystallized from MeOH-EtOAc to yield product, mp. 231-233°C (0.310 g). The filterate, on concentration, afforded second and third crops, mp. 231-232°C and 232-233°C respectively. Total yield of product (14), 0.730 g (11%) (lit.[82] mp. 232-233°C).

ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1720 (acid), 1650, 1520 (amide).

XV. N-Benzyloxycarbonyl L-Aspartic Acid Dimethyl Ester (ZAsp(β-OMe)OMe, 17):

(i) Aspartic acid dimethyl ester hydrochloride (Asp(β-OMe)OMe.HCl) :

To a refluxing suspension of L-Asp (5 g, 37.55 mmol) in dry MeOH (50 ml) was passed dry HCl for 2 h, the reaction mixture concentrated to ~ 10 ml, triturated with dry Et_2O(3 x 20 ml), filtered, crystallized from dry MeOH-Et_2O, filtered and washed with dry Et_2O to give 7.1 g (72%) of Asp(β-OMe)OMe.HCl, mp. 116°C (lit.[83] mp. 116-117°C).

ir : ν_{max}(KBr) cm^{-1} : 3450 (br), 2980 (br), 1750, 1640, 1595, 1505.

nmr : $\delta(CDCl_3)$: 3.3 (d, 2H, -CHC$\underline{H_2}$), 3.72, 3.82 (s, s, 6H, 2 x -COOC$\underline{H_3}$), 8.72 (br, 2H, -N$\underline{H_2}$).

(ii) ZAsp(β -OMe)OMe (17) :

To an ice-cooled and stirred solution of Asp(β -OMe)OMe.HCl (0.5 g, 2.78 mmol) in satd. aq. $NaHCO_3$ (40 ml) was added, in drops, Z-Cl (1.4 ml, 50% in toluene, 3.2 mmol) maintaining the pH between 8-9. The reaction mixture was left stirred overnight at rt, extracted with CH_2Cl_2 (3 x 30 ml), dried and evaporated to give 0.639 g (77%) of ZAsp(β -OMe)OMe,(17), as an oil.

ir : ν_{max}(neat) cm^{-1} : 3330 (-NH), 1710 (ester), 1495, 1430.

nmr : δ($CDCl_3$) : 2.9 (m, 2H, -CHC<u>H</u>$_2$), 3.6 (s, 3H, -COOC<u>H</u>$_3$), 3.7 (s, 3H, -COOC<u>H</u>$_3$), 4.55 (m, 1H, -C<u>H</u>), 5.05 (s, 2H, -OC<u>H</u>$_2$Ph), 5.65 (m, 1H, -N<u>H</u>), 7.25 (s, 5H, aromatic protons).

XVI. N-Acetyl L-Aspartic Acid Dimethyl Ester (AcAsp(β -OMe)OMe, 18) :

To an ice-cooled and stirred solution of Asp(β -OMe)OMe.HCl (1 g, 5.56 mmol) in satd. aq. $NaHCO_3$ (75 ml) was added Ac_2O (0.52 g, 5.56 mmol) maintaining the medium basic throughout. The reaction mixture was left stirred for 3 h, extracted with Et_2O (3 x 50 ml), dried, evaporated, and the residue on crystallization from dry PhH-Hexane gave 0.99 g (88%) of AcAsp(β -OMe)OMe, (18), mp. 56-57°C.

nmr : δ ($CDCl_3$) : 2.05 (s, 3H, -COC<u>H</u>$_3$), 2.95 (m, 2H, -CHC<u>H</u>$_2$), 3.7 (s, 3H, -COOC<u>H</u>$_3$), 3.75 (s, 3H, -COOC<u>H</u>$_3$), 4.9 (m, 1H, -C<u>H</u>), 6.45 - 6.95 (br, 1H, -N<u>H</u>).

XVII. N-Benzoyl L-Aspartic Acid Dimethyl Ester (BzAsp(β -OMe)OMe, 19) :

To an ice-cooled and vigorously shaken solution of Asp(β -OMe)OMe.HCl

(3.6 g, 20 mmol) in satd. aq. $NaHCO_3$ (250 ml) was added, in drops, Bz-Cl(2.84 g, 20 mmol), maintaining the medium basic throughout. The reaction mixture was left stirred for 3 h, extracted with Et_2O (3 x 50 ml), dried, solvents evaporated, and the residue on crystallization from PhH-Hexane gave 3.52 g (66%) of BzAsp(β -OMe)OMe, (19), mp. 89°C (lit.[51] mp. 96°C).

ir : ν_{max}(KBr) cm^{-1} : 3280 (-NH), 1725 (ester), 1640, 1520 (amide).

nmr : δ($CDCl_3$) : 3.1 (m, 2H, -CHC<u>H</u>$_2$), 3.7 (s, 3H, -COOC<u>H</u>$_3$), 3.8 (s, 3H, -COOC<u>H</u>$_3$), 5.1 (m, 1H, -C<u>H</u>), 7.2 - 8.0 (m, 6H, -N<u>H</u>, aromatic).

$[\alpha]_D^{25}$: +79.54 (c, 0.44, $CHCl_3$)

XVIII. N-Benzoyl L-Glutamic Acid Dimethyl Ester (BzGlu(γ -OMe)OMe, 20) :

(i) Glutamic acid dimethyl ester hydrochloride (Glu(γ -OMe)OMe.HCl) :

To a refluxing suspension of L-Glu (5 g, 34.2 mmol) in dry MeOH (75 ml) was passed dry HCl for 3 h, the reaction mixture cooled to rt and subjected to passage of dry HCl for another hour. The solvents were evaporated in vacuo below 40°C, the residue admixed with dry MeOH (20 ml) and evaporated to yield Glu(γ -OMe)OMe.HCl as a syrup. The crude product thus obtained was triturated with dry Et_2O(15 ml), filtered, washed with dry Et_2O(2 x 10 ml) and dried in vacuo over NaOH pellets to yield 5.24 g (73%) of product, mp. 115-116°C (lit.[84] mp. 116°C).

ir : ν_{max}(KBr) cm^{-1} 2980 (-NH), 1750 (ester), 1600, 1515 (amide).

(ii) BzGlu(γ -OMe)OMe (20) :

To an ice-cooled and vigorously shaken solution of Glu(γ -OMe)OMe.HCl

(0.212 g, 1 mmol) in satd. aq. $NaHCO_3$ (14 ml) was added Bz-Cl (0.16 g, 1.13 mmol), in drops, keeping the medium basic throughout. The reaction mixture was left stirred for 4 h, extracted with EtOAc (3 x 25 ml), dried, solvents evaporated in vacuo, and the residue on crystallization from EtOAc-Hexane gave 0.192 g (69%) of BzGlu(γ -OMe)OMe, (20), mp. 81°C (lit.[85] mp. 83°C).

ir : ν_{max}(KBr) cm^{-1} : 3260 (-NH), 1730 (ester), 1630, 1525 (amide).

nmr : $\delta(CDCl_3)$: 1.95 - 2.45 (m, 4H, $-CH(C\underline{H}_2)_2$), 3.55 (s, 3H, $-COOC\underline{H}_3$), 3.65 (s, 3H, $-COOC\underline{H}_3$), 4.5 - 4.95 (m, 1H, $-C\underline{H}$), 6.95 - 7.95 (m, 6H, $-N\underline{H}$, aromatic).

ms : m/z : 279 (M^+).

XIX. N-Benzoyl L-Tyrosinyl L-Phenylalanine Methyl Ester (BzTyr-PheOMe, 22) :

(i) N-Benzoyl tyrosine (BzTyr-OH) :

2N NaOH (12.5 ml) was added to a stirred solution of BzTyrOMe (4) (2.99 g, 10.5 mmol) in MeOH (12 ml), the reaction mixture left stirred overnight at rt, extracted with EtOAc(3 x 30 ml), the aqueous layer adjusted to pH ~2 with 2N HCl, saturated with NaCl, extracted with EtOAc(3 x 30 ml), dried and evaporated to yield 2.5 g (86%) of BzTyr-OH, mp. 165°C (lit.[86] mp. 165-166°C) which was used as such for the next experiment.

(ii) BzTyr-PheOMe (22) :

To a stirred solution of BzTyr-OH (1.43 g, 5.01 mmol) in dry DMF (12.5 ml) was added, in sequence, HOBt (0.676 g, 5.01 mmol), DCC (1.032 g, 5.01 mmol) and an ice-cold solution of L-phenylalanine methyl ester - freshly prepared by dropwise addition of dry Et_3N (1.04 ml, 7.5 mmol) to an ice-cold solution

of PheOMe.HCl (1.082 g, 5.01 mmol) in dry CH_2Cl_2 (12.5 ml) and then leaving stirred for 0.5 h. The reaction mixture was left stirred overnight at rt, filtered, the filterate extracted with $CHCl_3$(3 x 30 ml), the organic layer washed with 2N HCl, water (2 x 50 ml), 1 M Na_2CO_3(2 x 50 ml), dried, evaporated and crystallized from EtOAc-Hexane to yield 1.99 (89%) of BzTyr-PheOMe(22), mp. 205°C.

ir : ν_{max}(KBr) cm^{-1} : 3305, 1725 (ester), 1640, 1540 (amide).

nmr : δ($CDCl_3$ + DMSO-d_6) : 2.85 - 3.32 (m, 4H, 2 x -CHC<u>H</u>$_2$), 3.3 (s, 3H, -COOC<u>H</u>$_3$), 4.42 -4.87 (br, 1H, -C<u>H</u>), 5.22 - 5.72 (br, 1H, -C<u>H</u>), 6.5 - 8.1 (m, 16H, -N<u>H</u>, aromatic protons).

anal : Found : C, 69.90; H, 5.79; N, 6.25%

Calc. for $C_{26}H_{26}O_5N_2$: C, 69.96; H, 5.83; N, 6.28%.

XX. N-Benzoyl L-Tryptophyl L-Leucine Methyl Ester (BzTrp-LeuOMe, 23) :

(i) N-Benzoyl tryptophan (BzTrp-OH) :

To an ice-cooled and vigorously shaken solution of L-Trp (6.127 g, 30 mmol) in 2N NaOH (20 ml) was added Bz-Cl (4.5 ml, 33 mmol) and 2N NaOH (20 ml) in ten equal and alternate portions. The reaction mixture was left shaken for a further 0.25 h at rt, adjusted to pH~2 with cold conc. HCl, left aside at 4°C for 2 h, filtered, washed several times with ice-water, air-dried overnight, digested with boiling CCl_4(2 x 15 ml) to remove benzoic acid and crystallized from EtOH-H_2O to give 6.1 g (66%) of BzTrp-OH, mp. 105-106°C (lit.[87] mp. 104°C).

ir : ν_{max}(KBr) cm^{-1} : 3395, 1710 (acid), 1620, 1510 (amide).

(ii) Leucine methyl ester hydrochloride (LeuOMe.HCl) :

To a stirred suspension of L-Leu (5 g, 38.1 mmol) in dry MeOH (35 ml) was passed dry HCl at rt for 1 h, solvents evaporated under vacuo, and the residue on crystallization from dry MeOH-Et_2O gave 5.02 g (73%) of LeuOMe.HCl, mp. 149-150°C (lit.[88] mp. 151°C).

ir : ν_{max}(KBr) cm^{-1} : 3205 (-NH), 1740 (ester).

(iii) BzTrp-LeuOMe (23) :

To a stirred solution of BzTrp-OH (1.0 g, 3.24 mmol) in dry CH_2Cl_2 (6.5 ml) was added, in sequence, HOBt (0.44 g, 3.24 mmol), DCC (0.68 g, 3.24 mmol), followed by a solution of L-leucine methyl ester - freshly prepared by dropwise addition of dry Et_3N (0.6 ml, 4.33 mmol) to an ice-cooled and stirred solution of LeuOMe.HCl (0.60 g, 3.24 mmol) in dry CH_2Cl_2 (6.5 ml) and then leaving stirred for 0.5 h. The reaction mixture was left stirred overnight at rt, admixed with AcOH (3 drops), the precipitated DC urea filtered, washed with CH_2Cl_2, evaporated, dissolved in EtOAc (~50 ml), washed with 2N citric acid (3 x 15 ml), satd. aq. $NaHCO_3$ (3 x 15 ml), satd. NaCl, dried ($MgSO_4$) and evaporated to yield 1.25 g (89%) of nearly pure (23), (tlc), which was obtained as a gummy solid, which could not be crystallized.

ir : ν_{max}(neat) cm^{-1} : 3260 (-NH), 1715 (ester), 1620, 1510 (amide).

nmr : $\delta(CDCl_3)$: 0.8 (br, 6H, $-CH(C\underline{H}_3)_2$), 1.45 (m, 2H, $-CHC\underline{H}_2CH$), 2.2 (br s, 1H, $-C\underline{H}(CH_3)_2$), 3.3 (d, 2H, $-CHC\underline{H}_2$), 3.6 (s, 3H, $-COOC\underline{H}_3$), 4.5 (m, 1H, -C<u>H</u>), 5.0 (m, 1H, -C<u>H</u>), 6.5 - 8.0 (m, 12H, -N<u>H</u>, aromatic protons), 8.5 (br s, 1H, -N<u>H</u>).

ms : m/z : 435 (M^+).

anal : Found : C, 68.91; H, 6.61; N, 9.54%

Calc. for $C_{25}H_{29}O_4N_3$: C, 68.97; H, 6.67; N, 9.66%

XXI. N-Benzoyl L-Tryptophyl L-Phenylalanine Methyl Ester (BzTrp-PheOMe, 24) :

To a stirred solution of BzTrp-OH (0.998 g, 3.24 mmol) in dry CH_2Cl_2 (6.5 ml) was added, in sequence, HOBt (0.437 g, 3.24 mmol), DCC (0.680 g, 3.24 mmol), followed by a solution of L-phenylalanine methyl ester - freshly prepared by dropwise addition of dry Et_3N (0.60 ml, 4.33 mmol) to an ice-cooled and stirred solution of PheOMe.HCl (0.699 g, 3.24 mmol) in dry CH_2Cl_2 (6.5 ml) and leaving aside for 0.5 h. Work up precisely as described in Experiment XIX yielded 1.43 g (94%) of BzTrp-PheOMe, (24), as a gummy solid.

ir : ν_{max}(neat) cm^{-1} : 3270 (-NH), 1720 (ester), 1625, 1510 (amide).

nmr : $\delta(CDCl_3)$: 2.8 (m, 2H, -CHC$\underline{H_2}$Ph), 3.2 (m, 2H, -CHC$\underline{H_2}$), 3.6 (s, 3H, -COOC$\underline{H_3}$), 4.9 (m, 2H, 2 x -C$\underline{H}$), 6.4 - 7.8 (m, 17 H, -N$\underline{H}$, aromatic protons), 8.4 (br s, 1H, -N$\underline{H}$).

ms : m/z : 469 (M^+), 470 (M^++1)

anal : Found : C, 71.56; H, 5.68; N, 8.72%

Calc. for $C_{28}H_{27}O_4N_3$: C, 71.64; H, 5.76; N, 8.96%

XXII. N-Benzoyl L-Prolyl L-Phenylalanine Methyl Ester (BzPro-PheOMe, 25) :

(i) N-Benzoyl proline (BzPro-OH) :

To a vigorously shaken solution of L-Pro (8.88 g, 77.2 mmol) in 1.5 N NaOH (50 ml) cooled in an ice-salt mixture was added, alternately, in ten lots each, Bz-Cl (12.6 ml, 92.6 mmol) and 2N NaOH (300 ml), keeping the medium

XXIII. N-Benzoyl L-Phenylalanyl L-Proline Methyl Ester (BzPhe-ProOMe, 26) :

(i) N-Benzoyl phenylalanine (BzPhe-OH) :

To an ice-salt cooled and vigorously shaken solution of L-Phe (8.259 g, 0.05 mol) in 2N NaOH (75 ml) was added, over 0.5 h, Bz-Cl (8.26 g, 0.05 mol). The reaction mixture was left stirred at 0°C for 0.5 h, then at rt for 3 h, adjusted to pH 2 with conc. HCl, left aside at 0°C for 2-3 h, filtered, the residue washed with hot CCl_4(3 x 20 ml) and dried to yield 13.2 g (98%) of BzPhe-OH, mp 145-146°C (lit.[89] mp. 145-146°C).

ir : ν_{max}(KBr) cm^{-1} : 3280, 1700 (acid), 1630, 1515 (amide).

(ii) BzPhe-ProOMe (26) :

To a stirred solution of BzPhe-OH (1 g , 3.7 mmol) in dry CH_2Cl_2 (9.5 ml) was added, in sequence, HOBt (0.5 g, 3.7 mmol), DCC (0.777 g, 3.7 mmol), followed by a solution of L-proline methyl ester - freshly prepared by dropwise addition of dry Et_3N(0.7 ml, 4.8 mmol) to an ice-cooled and stirred solution of ProOMe.HCl (0.612 g, 3.7 mmol) in dry CH_2Cl_2 (9.5 ml) and then leaving aside for 0.5 h. Work up precisely as described in Experiment XIX gave 1.02 g (73%) of BzPhe-ProOMe,(26),as a gummy solid.

ir : ν_{max}(neat) cm^{-1} : 3300 (-NH), 1735 (ester), 1625, 1525 (amide).

nmr : $\delta(CDCl_3)$: 1.5 - 2.1 (br, 4H, $-CH(C\underline{H}_2)_2$), 3.05 (d, 2H, $-CHC\underline{H}_2Ph$), 3.6 (m, 5H, $-NC\underline{H}_2(CH_2)_2$, $-COOC\underline{H}_3$), 4.25 (br, 1H, $-C\underline{H}$), 5.05 (m, 1H, $-C\underline{H}$), 6.9 - 7.9 (m, 11H, $-N\underline{H}$, aromatic protons).

ms : m/z : 380 (M^+), 381 (M^++1).

XXIV. N-Benzoyl L-Aspartyl L-Aspartic Acid Trimethyl Ester (BzAsp(β -OMe)-Asp(β -OMe)OMe, 27) :

(i) Aspartic acid β -methyl ester hydrochloride (Asp(β -OMe)OH.HCl) :

To ice-cooled and stirred dry MeOH (25 ml) was added $SOCl_2$(3.85 ml, 52.5 mmol) followed by L-Asp (5g, 37.5 mmol) in lots. The reaction mixture was allowed to attain rt, left stirred for 0.5h, admixed with dry Et_2O filtered and dried to yield 5.32 g (77%) of Asp(β -OMe)OH.HCl, mp. 192-193°C (lit.[90] mp. 190°C).

(ii) N-Benzoyl aspartic acid β -methyl ester (BzAsp(β -OMe)-OH) :

To an ice-cooled and vigorously shaken solution of Asp(β -OMe)OH.HCl (12 g, 65.2 mmol) in satd. aq. $NaHCO_3$ (600 ml) was added, in drops, Bz-Cl (9 ml, 76.8 mmol), maintaining the medium basic throughout. The reaction mixture was left stirred for 2 h, cooled, adjusted to pH~2 with 2N HCl (~130 ml), saturated with NaCl, extracted with Et_2O(3 x 100 ml), dried, solvents evaporated and the residue on crystallization from dry PhH gave 12.42 g (76%) of BzAsp-(β -OMe)-OH, mp. 126-127°C (lit.[91] mp. 125-126°C).

ir : ν_{max}(KBr) cm^{-1}: 3320 (-NH), 1750 (ester), 1720 (acid), 1630, 1530 .

(iii) BzAsp(β -OMe)-Asp(β -OMe)OMe (27) :

To a stirred solution of BzAsp(β -OMe)-OH (0.251 g, 1 mmol) in dry CH_2Cl_2 (2.5 ml) was added, in sequence, HOBt (0.135 g, 1 mmol), DCC (0.210 g, 1 mmol), followed by a solution of L-aspartic acid dimethyl ester - freshly prepared by dropwise addition of dry Et_3N (0.2 ml, 1.3 mmol) to an ice-cooled and stirred solution of Asp(β -OMe)OMe.HCl (0.198 g, 1 mmol) in

dry CH_2Cl_2 (2.5 ml) and then leaving aside for 0.5 h. The reaction mixture was left stirred overnight at rt and work up, precisely as described in Experiment-XIX, gave 0.269 g (68%) of BzAsp(β -OMe)-Asp(β -OMe)OMe, mp. 138-139°C (lit.[51] mp. 138-139°C).

ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1750 (ester), 1650, 1550 (amide).

nmr : $\delta(CDCl_3)$: 2.7-3.1 (m, 4H, 2 x -CHC<u>H</u>$_2$), 3.6 (s, 3H, -COOC<u>H</u>$_3$), 3.7 (s, 6H, 2 x -COOC<u>H</u>$_3$), 4.6-5.25 (br, 2H, 2 x -C<u>H</u>), 6.95-8.0 (m, 7H, -N<u>H</u>, aromatic protons).

XXV. <u>N-Benzoyl L-Aspartyl L-Leucine Dimethyl Ester (BzAsp(β -OMe)-LeuOMe, 28)</u> :

To a stirred solution of BzAsp(β -OMe)-OH (0.251 g, 1 mmol) in dry CH_2Cl_2 (2.5 ml) was added, in sequence, HOBt (0.135 g, 1 mmol), DCC (0.210 g, 1 mmol), followed by a solution of L-leucine methyl ester - freshly prepared by dropwise addition of dry Et_3N (0.2 ml, 1.3 mmol) to an ice-cooled and stirred solution of LeuOMe.HCl (0.182 g, 1 mmol) in dry CH_2Cl_2(2.5 ml) and then leaving aside for 0.5 h. The reaction mixture was left stirred overnight at rt and work up precisely as described in Experiment XIX gave 0.313 g (83%) of BzAsp(β -OMe)-LeuOMe, (<u>28</u>), as a gummy solid.

ir : ν_{max}(neat) cm^{-1} : 3260, 1720 (ester), 1620, 1510 (amide).

nmr : $\delta(CDCl_3)$: 0.9 (br, 6H, -CH(C<u>H</u>$_3$)$_2$), 1.6 (br, 2H, -C<u>H</u>$_2$CH(CH$_3$)$_2$), 2.5 (m, 1H, -C<u>H</u>(CH$_3$)$_2$), 2.9 (m, 2H, -C<u>H</u>$_2$COOCH$_3$), 3.7 (s, s, 6H, 2 x -COOC<u>H</u>$_3$), 4.5 (br, 1H, -C<u>H</u>), 5.0 (br, 1H, -C<u>H</u>), 6.7 -8.3 (m, 7H, -N<u>H</u>, aromatic protons).

ms : m/z : 378 (M^+), 379 (M^++1).

anal : Found : C, 60.39; H, 6.80; N, 7.33%

Calc. for $C_{19}H_{26}O_6N_2$: C, 60.32; H, 6.88; N, 7.41%

XXVI. N-Benzoyl L-Aspartyl L-Phenylalanine Dimethyl Ester (BzAsp(β -OMe)-PheOMe, 29) :

To a stirred solution of BzAsp(β -OMe)-OH (0.251 g, 1 mmol) in dry CH_2Cl_2 (2.5 ml) was added, in sequence, HOBt (0.135 g, 1 mmol), DCC (0.210 g, 1 mmol), followed by a solution of L-phenylalanine methyl ester - freshly prepared by dropwise addition of dry Et_3N (0.2 ml, 1.3 mmol) to an ice-cooled and stirred solution of PheOMe.HCl (0.216 g, 1 mmol) in dry CH_2Cl_2 (2.5 ml) and then leaving aside for 0.5 h. The reaction mixture was left stirred overnight at rt and work up, precisely as described in Experiment XIX, gave 0.349 g (85%) of BzAsp(β -OMe)-PheOMe,(29), mp. 115-117°C (dry EtOAc-Hexane).

ir : ν_{max}(KBr) cm^{-1} : 3260, 1730 (ester), 1625, 1520 (amide).

nmr : δ($CDCl_3$) : 2.7-3.25 (m, 4H, 2 x -CHC<u>H</u>$_2$), 3.75 (s, 6H, 2 x -COOC<u>H</u>$_3$), 4.9 (m, 2H, 2 x -C<u>H</u>), 6.6-7.9 (m, 12H, -N<u>H</u>, aromatic protons).

ms : m/z : 412 (M^+), 413 (M^++1).

anal : Found : C, 64.12; H, 5.79; N, 6.71%

Calc. for $C_{22}H_{24}O_6N_2$: C, 64.08; H, 5.83; N, 6.80%

XXVII. N-Benzoyl L-Prolyl L-Aspartic Acid Dimethyl Ester (BzPro-Asp(β -OMe)-OMe, 30) :

To a stirred solution of BzPro-OH (0.219 g, 1 mmol) in dry CH_2Cl_2

(2.5 ml) was added, in sequence, HOBt (0.135 g, 1 mmol), DCC (0.210 g, 1 mmol), followed by a solution of L-aspartic acid dimethyl ester - freshly prepared by dropwise addition of dry Et_3N (0.2 ml, 1.3 mmol) to an ice-cooled and stirred solution of Asp(β -OMe)OMe.HCl (0.198 g, 1 mmol) in dry CH_2Cl_2 (2.5 ml) and then leaving aside for 0.5 h. The reaction mixture was left stirred overnight at rt and work up, precisely as described in Experiment XIX, gave 0.326 g of crude product which on chromatography over silica gel and elution with PhH: EtOAc :: 3:7 gave 0.23 g (63%) of BzPro-Asp(β -OMe)OMe, (30), as a gummy solid.

ir : ν_{max}(neat) cm^{-1} : 3300 (-NH), 1735 (ester), 1675, 1620, 1520 (amide).

nmr : δ($CDCl_3$) : 1.8-2.6 (br, 4H, -CH($C\underline{H}_2)_2$), 2.9 (d, 2H, -CH$C\underline{H}_2$COOCH_3), 3.5-3.6 (br, 2H, -$(CH_2)_2C\underline{H}_2$); 3.65 (s, 3H, -COO$C\underline{H}_3$), 3.75 (s, 3H, -COO$C\underline{H}_3$), 4.8 (m, 2H, 2 x -C$\underline{H}$), 7.4 (m, 6H, -N$\underline{H}$, aromatic).

ms : m/z : 362 (M^+), 363 (M^++1).

anal : Found : C, 59.76; H, 6.02; N, 7.66%

Calc. for $C_{18}H_{22}O_6N_2$: C, 59.67; H, 6.08; N, 7.73%

XXVIII. N-Benzoyl L-Glutamyl L-Aspartic Acid Trimethyl Ester (BzGlu(γ -OMe)-Asp(β -OMe)OMe, 31) :

(i) Glutamic acid γ -methyl ester (Glu(γ -OMe)-OH) :

To ice-cooled dry MeOH (120 ml) was added, in drops, $SOCl_2$ (7.2 ml, 0.1 mol). The reaction mixture was allowed to attain rt, admixed with L-Glu (14.7 g, 0.1 mol), stirred at rt for 0.5 h, cooled to 0-5°C, admixed with, over

5 min, Et_3N (35 ml, 0.25 mol), filtered and dried to yield 13 g (81%) of Glu(γ-OMe)-OH, mp. 183°C (lit.[92] mp. 182°C).

(ii) N-Benzoyl glutamic acid γ -methyl ester (BzGlu(γ -OMe)-OH) :

To an ice-cooled and stirred solution of Glu(γ -OMe)-OH (2.64 g, 16.5 mmol) in satd. aq. $NaHCO_3$ (175 ml) was added, in drops, Bz-Cl (2.25 ml, 19.2 mmol), maintaining the medium basic throughout. The reaction mixture was left stirred at rt for 3 h, cooled, adjusted to pH~2 with 2N HCl (~60 ml), saturated with NaCl, extracted with EtOAc (3 x 25 ml), dried and evaporated to yield 2.89 g (66%) of BzGlu(γ -OMe)-OH, mp. 106-107°C (dry Acetone-Hexane) (lit.[93] mp. 107°C).

ir : ν_{max}(KBr) cm^{-1} : 3340 (-NH), 1735 (br), 1650, 1545 (amide).

(iii) BzGlu(γ -OMe)-Asp(β -OMe)OMe (31) :

To a stirred solution of BzGlu(γ -OMe)-OH (0.264 g, 1 mmol) in dry CH_2Cl_2 (2.5 (ml) was added, in sequence, HOBt (0.135 g, 1 mmol), DCC (0.210 g, 1 mmol), followed by a solution of L-aspartic acid dimethyl ester - freshly prepared by dropwise addition of dry Et_3N (0.2 ml, 1.3 mmol) to an ice-cooled and stirred solution of Asp(β -OMe)OMe.HCl (0.198 g, 1 mmol) in dry CH_2Cl_2 (2.5 ml) and then leaving aside for 0.5 h. The reaction mixture was left stirred overnight at rt and work up, precisely as described in Experiment XIX, gave 0.306 g of crude product which on chromatography over silica gel and elution with PhH : EtOAc :: 4:6 gave 0.213 g (54%) of BzGlu(γ -OMe)-Asp(β -OMe)OMe,(31),as a gummy solid.

ir : ν_{max}(neat) cm^{-1} : 3310 (-NH), 1730 (ester), 1630, 1525 (amide).

nmr : δ ($CDCl_3$) : 2.1-2.75 (m, 4H, -CH($C\underline{H}_2$)$_2$), 2.9 (m, 2H, -CH$C\underline{H}_2$), 3.65 (s, s, 6H, 2 x -COO$C\underline{H}_3$), 3.75 (s, 3H, -COO$C\underline{H}_3$), 4.55-5.2 (m, 2H, 2 x -C$\underline{H}$), 7.1-8.1 (m, 7H, -N$\underline{H}$, aromatic protons).

ms : m/z : 408 (M^+), 409 (M^++1).

anal : Found : C, 55.79; H, 5.91; N, 6.79%

Calc. for $C_{19}H_{24}O_8N_2$: C, 55.88; H, 5.88; N, 6.86%

XXIX. Reaction of N-Benzyloxycarbonyl L-Phenylalanine Methyl Ester (ZPheOMe, 1) with Ruthenium Tetroxide (RuO_4) : Isolation of N-Benzyloxycarbonyl L-Aspartic Acid α-Methyl Ester (ZAsp(β -OH)OMe, 32) :

A solution of ZPheOMe (1.2 g, 3.83 mmol) in MeCN (15.3 ml) was admixed with CCl_4:H_2O :: 1:2 (46 ml), $NaIO_4$ (14.75 g, 69 mmol), $RuCl_3.3H_2O$ (0.029 g, 2.2 mol%), sealed, left shaken for 60 h at rt, cautiously opened, filtered, the residue triturated with CCl_4 (3 x 10 ml), the aqueous layer washed with EtOAc (3 x 30 ml), the combined organic extracts dried, evaporated, the resulting residue (1.087 g) digested for 3 h with satd. aq. $NaHCO_3$(~40 ml), extracted with EtOAc (3 x 25 ml), dried and evaporated to afford 0.093 g of intractable neutral residue.

The bicarbonate extract was cooled in ice, adjusted to pH~3 with 2N H_2SO_4, saturated with NaCl, extracted with EtOAc (3 x 25 ml), dried and evaporated to give ZAsp(β -OH)OMe, (32), (0.911 g, 85%) as a gummy solid (lit.[94] mp. 159-160°C).

nmr : δ ($CDCl_3$) : 3.0 (m, 2H, -CH$C\underline{H}_2$), 3.7 (s, 3H, -COO$C\underline{H}_3$), 5.1 (s, 2H, -O$C\underline{H}_2$Ph), 4.6 (m, 1H, -C$\underline{H}$), 5.8 (m, 1H, -N$\underline{H}$), 7.2 (s, 5H, aromatic).

compound (32) was further characterized as it's dimethyl ester via addition of excess ethereal CH_2N_2 to a methanolic solution followed by evaporation of solvents. The dimethyl ester thus obtained was identical in all respects to that of the authentic sample, ZAsp(β -OMe)OMe, (17), (Experiment XV).

XXX. Reaction of N-Acetyl L-Phenylalanine Methyl Ester (AcPheOMe, 2) with RuO_4 : Isolation of N-Acetyl L-Aspartic Acid α -Methyl Ester (AcAsp(β -OH)OMe, 33) :

A solution of AcPheOMe (0.8 g, 3.62 mmol) in MeCN (14.5 ml) was admixed with CCl_4: H_2O :: 1:2 (43.5 ml), $NaIO_4$ (13.937 g, 65.16 mmol), $RuCl_3.3H_2O$ (0.027 g, 2.2 mol%) and left shaken for 60 h at rt. Work up, precisely as described in Experiment XXIX gave, from the bicarbonate extract, 0.477 g (70%) of AcAsp(β -OH)OMe, (33), which was identified via esterification with CH_2N_2 and comparison with an authentic sample of AcAsp(β -OMe)OMe, (18).

XXXI. Reaction of N-Benzyloxycarbonyl Glycine Methyl Ester (ZGlyOMe, 3) with RuO_4 : A test for the susceptibility of the benzyloxycarbonyl group towards Ru^{VIII} :

A solution of ZGlyOMe (0.650 g, 2.9 mmol) in MeCN (12 ml) was admixed with CCl_4: H_2O :: 1:2 (36 ml), $NaIO_4$(11.55 g, 54 mmol) and $RuCl_3.3H_2O$ (0.023 g, 2.2 mol%) and left shaken for 60 h at rt. Work up, exactly as described in Experiment XXIX, followed by careful analysis of the resulting product indicated total recovery of the starting material.

Reaction of N-Benzoyl L-Tyrosine Methyl Ester (BzTyrOMe, 4) with Ru^{VIII}:

Isolation of N-Benzoyl L-Aspartic Acid α-Methyl Ester (BzAsp(β -OH)OMe, 34) :

The reaction of BzTyrOMe (4) (1.5 g, 5 mmol) with Ru^{VIII}, as described in the previous experiments, followed by work up yielded 1.13 g (85%) of BzAsp(β -OH)OMe, mp. 127-128°C (dry EtOAc-Hexane).

ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1745 (ester), 1710 (acid), 1620, 1520 (amide).

nmr : δ ($CDCl_3$) (400 MHz) : 3.05, 3.2 (dd, dd, 2H, -CHC$\underline{H_2}$), 3.85 (s, 3H, -COOC$\underline{H_3}$), 5.05 (m, 1H, -C$\underline{H}$), 7.2-7.8 (m, 6H, -N$\underline{H}$, aromatic).

^{13}C-NMR: δ($CDCl_3$+DMSO-d_6)(400MHz): 171.7, 170.7, 166.2 (3 x -$\underline{C}$O), 133.1, 130.8, 127.6, 126.5 (Ph ring carbons), 51.6 (-$\underline{C}$H), 48.5 (-$\underline{C}H_3$), 35.3 (-$\underline{C}H_2$).

ms : m/z : 251 (M^+).

anal : Found : C, 56.75; H, 5.30; N, 5.50%

Calc. for $C_{12}H_{13}O_5N$: C, 57.37; H, 5.18; N, 5.58%

The structural assignment for (34) was further confirmed via transformation to BzAsp(β -OMe)OMe (19) with CH_2N_2 and comparison of the product with that of an authentic sample.

XXXIII. Oxidation of N-Benzoyl L-Tyrosine Methyl Ester (BzTyrOMe, 4) with Ru^{VIII} under restricted duration : Isolation of N-Benzoyl L-Aspartic Acid α -Methyl Ester (BzAsp(β -OH)OMe, 34) :

The oxidation of BzTyrOMe, (4), (0.75 g, 2.5 mmol) carried out as

described in the previous experiment, but with a restricted duration of 12 h, yielded, after work up, 0.454 g (69%) of BzAsp(β-OH)OMe, mp. 125°C, which was further identified via transformation to the dimethyl ester (19) and comparison with an authentic sample.

XXXIV. Reaction of N-Benzoyl L-Tyrosine Methyl Ester (BzTyrOMe, 4) with RuO_4 at pH 3 : Isolation of N-Benzoyl L-Aspartic Acid α-Methyl Ester (BzAsp(β-OH)OMe, 34) :

A solution of BzTyrOMe (1.2 g, 4 mmol) in MeCN (16 ml) was admixed with CCl_4: pH 3 Phosphate buffer :: 1:2 (48 ml), $NaIO_4$ (15.4 g, 72 mmol), $RuCl_3 \cdot 3H_2O$ (0.030 g, 2.2 mol%), stirred for 18 h at rt, the pH of the medium adjusted to 6-7 with satd. aq. $NaHCO_3$ and filtered. The organic layer was evaporated and the resulting residue (1.196 g) on digestion with satd. aq. $NaHCO_3$ (40 ml), removal of small amounts of neutral material with EtOAc, followed by adjustment of pH to ~2 and work up as described previously gave 0.832 g (83%) of BzAsp-(β-OH)OMe, (34), mp. 125-126°C (dry EtOAc-Hexane) which was further characterized via transformation to BzAsp(β-OMe)OMe, (19), and comparison with an authentic sample (Experiment XVII).

XXXV. Reaction of N-Benzoyl L-Tyrosine Methyl Ester (BzTyrOMe, 4) with Ru^{VIII} at pH 9 : Isolation of N-Benzoyl L-Aspartic Acid α-Methyl Ester (BzAsp(β-OH)OMe, 34) :

A solution of BzTyrOMe (1.2 g, 4 mmol) in MeCN (16 ml) was admixed with CCl_4: satd. aq. $NaHCO_3$(pH~9) :: 1:2 (48 ml), $NaIO_4$(15.4 g, 72 mmol), $RuCl_3 \cdot 3H_2O$ (0.030 g, 2.2 mol%), left stirred overnight at rt, filtered, extracted

with EtOAc (3 x 25 ml) to remove small amounts of neutral material, adjusted to pH~3 with 2N H_2SO_4, saturated with NaCl, extracted with EtOAc (3 x 30 ml), dried and evaporated to yield 0.838 g (83%) of BzAsp(β -OH)OMe, (34), mp. 125°C (dry EtOAc-Hexane) which was identical with that obtained from previous experiments.

XXXVI. Reaction of N-Benzyloxycarbonyl L-Tryptophan Methyl Ester (ZTrpOMe, 5) with Ru^{VIII} : Isolation of N-Benzyloxycarbonyl L-Aspartic Acid α-Methyl Ester (ZAsp(β -OH)OMe, 32) and Benzyl Carbamate :

A mixture of ZTrpOMe, (5), (1.5 g, 4.26 mmol) in MeCN (17 ml) was admixed with CCl_4 : H_2O :: 1:2 (51 ml), $NaIO_4$ (16.4 g, 76.7 mmol) and $RuCl_3.3H_2O$ (0.033g, 2.2 mol%), sealed, left shaken for 60 h at rt and worked up, as described in Experiment XXIX. The neutral extract yielded, on crystallization from dry PhH-Hexane, 0.203 g (32%) of benzyl carbamate, mp. 87°C, identical to that of an authentic sample.

The bicarbonate extract, on adjustment to pH~3 followed by work up as described previously, gave, 0.82 g (85%) of ZAsp(β -OH)OMe which was further characterized by transformation to its dimethyl ester (17) and comparison with an authentic sample.

XXXVII. Reaction of N-Benzoyl L-Tryptophan Methyl Ester (BzTrpOMe, 6) with Ru^{VIII} : Isolation of N-Benzoyl L-Aspartic Acid α -Methyl Ester (BzAsp-(β -OH)OMe, 34), N^{α}-Benzoyl N^{ω}-Formylkynurenine Methyl Ester (N^{α}-Bz N^{ω}-ForKyn-OMe, 35) and Benzamide :

A mixture of BzTrpOMe, (6), (1.6 g, 5 mmol), MeCN (20 ml), CCl_4

(20 ml), H_2O (40 ml), $NaIO_4$ (19.26 g, 90 mmol) and $RuCl_3.3H_2O$ (0.038 g, 2.2 mol%) was sealed, left shaken for 60 h at rt, opened, filtered, the residue triturated with CCl_4 (3 x 10 ml), the aqueous layer extracted with EtOAc (3 x 20 ml), the combined organic extracts dried, evaporated, the resulting residue (1.3 g) digested with satd. aq. $NaHCO_3$ (40 ml) for 3 h, extracted with EtOAc (3 x 25 ml), dried and evaporated to afford 0.4 g of neutral residue which, on chromatography on silica gel and elution with PhH : EtOAc :: 7:3, gave 0.244 g (14%) of N^{α}-Bz N^{ω}-ForKyn-OMe, (35), mp. 94-95°C (dry EtOAc-Hexane).

ir : ν_{max}(KBr) cm^{-1} : 3310 (-NH), 1750 (ester), 1730 (-CO), 1690, 1655, 1630, 1580, 1510.

nmr : $\delta(CDCl_3)$: 3.85 (s+d, 5H, $-COOC\underline{H}_3$+ $-CHC\underline{H}_2$), 5.15 (m, 1H, $-C\underline{H}$), 7.05-8.05 (m, 9H, aromatic protons), 8.65 (br, 2H, 2 x $-N\underline{H}$), 11.3 (br, 1H, $-C\underline{H}O$).

^{13}C-NMR : $\delta(CDCl_3)$(100 MHz) : 200.0, 171.6, 166.1, 161.1 (4 x $-\underline{C}O$), 134.5, 134.0 133.5, 131.3, 130.6, 129.6, 128.1, 127.1, 123.2, 122.7, 115.9 (phenyl ring carbons), 51.9 ($\underline{C}H$), 48.6 ($\underline{C}H_3$), 40.9 ($\underline{C}H_2$).

ms : m/z : 354 (M^+).

anal : Found : C, 64.58; H, 5.12%

Calc. for $C_{19}H_{18}O_5N_2$: C, 64.41; H, 5.08%

Further elution with PhH : EtOAc :: 1:1 gave 0.08 g (13%) of benzamide, mp. 127-128°C, identical with that of an authentic sample.

The bicarbonate extract was adjusted to pH~3 with 2N H_2SO_4, saturated with NaCl, extracted with EtOAc (3 x 25 ml), dried and evaporated

to yield 0.810 g (65%) of BzAsp(β -OH)OMe , (34), mp. 125-127°C, identical to samples obtained from previous experiments.

Interestingly, the above reaction, when carried out in Summer (rt~ 35°C), gave 77% of BzAsp(β -OH)OMe, (34), 10% of $BzNH_2$ and none of N^{α}-Bz N^{ω} -For-Kyn-OMe , (35).

XXXVIII. Reaction of N-Benzoyl L-Tryptophan Methyl Ester (BzTrpOMe, 6) with Ru^{VIII} under restricted time duration : Isolation of N-Benzoyl L-Aspartic Acid α -Methyl Ester (BzAsp(β -OH)OMe, 34) and N^{α}-Benzoyl N^{ω}-Formylkynurenine Methyl Ester (N^{α}-Bz N^{ω}-ForKyn-OMe, 35) in enhanced yields :

A mixture of BzTrpOMe (0.805 g, 2.5 mmol), MeCN (10 ml), CCl_4 (10 ml), H_2O (20 ml), $NaIO_4$ (9.63 g, 45 mmol), and $RuCl_3 . 3H_2O$ (0.019 g, 2.2 mol%) was sealed, shaken for 12 h at rt and worked up precisely as described in the previous experiment to yield 0.18 g (20%) of N^{α}-Bz N^{ω}-ForKyn-OMe, (35), mp. 93-95°C and 0.39 g (62%) of BzAsp(β -OH)OMe, (34), mp. 125-127°C.

XXXIX. Reaction of N-Benzoyl L-Tryptophan Methyl Ester (BzTrpOMe, 6), with Ru^{VIII} using restricted quantity of periodate : Isolation of N^{α}-Benzoyl N^{ω}-Formylkynurenine Methyl Ester (N^{α}-Bz N^{ω}-ForKyn-OMe, 35) :

The oxidation of BzTrpOMe (0.805 g, 2.5 mmol) with restricted amount of $NaIO_4$ (1.07 g, 5 mmol) in MeCN : CCl_4 : H_2O (10 : 10 : 20 ml) and $RuCl_3 . 3H_2O$ (0.019 g, 2.2 mol%) as described in the previous experiment and work up gave 0.35 g (47%) of N^{α}-Bz N^{ω}-ForKyn-OMe, (35), mp. 93-95°C. No BzAsp(β -OH)OMe , (34) could be obtained in this experiment.

XL. Reaction of N-Benzoyl L-Tryptophan Methyl Ester (BzTrpOMe, 6) with Ozone : Isolation of N^{α}-benzoyl N^{ω}-Formylkynurenine Methyl Ester (N^{α}-Bz N^{ω}-ForKyn-OMe, 35) :

A stream of ozone was bubbled through a solution of BzTrpOMe (1 g, 3.1 mmol) in glacial AcOH (20 ml) at 20°C for 4 h, the resulting mixture poured onto ice-water (~200 ml), extracted with EtOAc (3 x 50 ml), washed with satd. aq. $NaHCO_3$ (2 x 50 ml), dried, evaporated, and the residue (0.967 g) on chromatography on silica gel and elution PhH : EtOAc :: 7:3 gave 0.30 g of unchanged starting material and 0.445 g (58%) of N^{α}-Bz N^{ω}-ForKyn-OMe, (35), mp. 93-95°C, which was identical to that obtained previously.

XLI. Reaction of N^{α}-Benzoyl N^{ω}-Formylkynurenine Methyl Ester (N^{α}-Bz N^{ω}-ForKyn-OMe, 35) with Ru^{VIII}: Isolation of N-Benzoyl L-Aspartic Acid α-Methyl Ester (BzAsp-(β-OH)OMe, 34) :

A mixture of N^{α}-Bz N^{ω}-ForKyn-OMe, (35), (0.1 g, 0.282 mmol), MeCN (1.5 ml), CCl_4 (1.5 ml), H_2O (3 ml), $NaIO_4$ (1.087 g, 5.08 mmol) and $RuCl_3.3H_2O$ (0.002 g, 2.2 mol%) was sealed, left shaken for 60 h and worked up as described previously to yield 0.027 g of unchanged starting material and 0.050 g (97%) of BzAsp(β-OH)OMe, (34), mp. 124-126°C, identical with samples obtained previously.

XLII. Reaction of Tetrahydrocarbazole with Ru^{VIII} : Isolation of adipic acid :

A mixture of tetrahydrocarbazole (0.855 g, 5 mmol), MeCN (20 ml), CCl_4 (20 ml), H_2O (40 ml), $NaIO_4$ (19.25 g, 90 mmol) and $RuCl_3.3H_2O$ (0.038 g, 2.2 mol%) was sealed and left shaken for 60 h at rt. Work up as descri-

bed previously gave 0.45 g (61%) of adipic acid which was further characterized via transformation to the dimethyl ester and comparison with an authentic sample; bp. 120°/14 mm.

XLIII. The Reaction of L-Valine with Ru^{VIII} : Isolation of Isobutyric Acid :

A solution of L-valine (0.468 g, 4 mmol), in MeCN (16 ml) was admixed with CCl_4: H_2O (1:2, 48 ml), $NaIO_4$ (15.4 g, 72 mmol) and $RuCl_3.3H_2O$ (0.030 g, 2.2 mol%), sealed and left shaken for 60 h at rt. Work up as described in previous experiments gave 0.280 g of acidic product which, on bulb to bulb distillation, gave 0.232 g (66%) of isobutyric acid, bp. 145-155°C, whose properties were identical with that of an authentic sample.

XLIV. The Reaction of L-Phenylalanine with Ru^{VIII} : Isolation of Phenylacetic Acid :

A solution of L-phenylalanine (0.661 g, 4 mmol), in MeCN (16 ml) was admixed with CCl_4: H_2O (1:2, 48 ml), $NaIO_4$ (15.4 g, 72 mmol) and $RuCl_3.3H_2O$ (0.030 g, 2.2 mol%), sealed and left shaken for 8 h at rt. Work up as described in previous experiments gave 0.337 g of acidic product which was extracted with small amounts of hot dry benzene to remove 0.06 g (13%) of benzoic acid. The benzene extract on evaporation gave 0.234 g (43%) of phenylacetic acid mp. 76-77°C, whose properties were identical with that of an authentic sample.

XLV. Reaction of N^{α}-Benzyloxycarbonyl L-Histidine Methyl Ester (ZHisOMe, 7) with Ru^{VIII} : Isolation of N-Benzyloxycarbonyl Aspartoyl Urea α-Methyl Ester (ZAsn(β-$CONH_2$)OMe, 36) and N-benzyloxycarbonyl L-Aspartic

Acid α-Methyl Ester (ZAsp(β-OH)OMe, 32) :

A mixture of ZHisOMe, (7), (1.5 g, 4.95 mmol), MeCN (20 ml), CCl_4 (20 ml), H_2O (40 ml), $NaIO_4$ (29.058 g, 89.1 mmol) and $RuCl_3.3H_2O$ (0.037 g, 2.2 mol%) was sealed, left shaken for 60 h, filtered, the residue washed with CCl_4 (3 x 10 ml), the aqueous layer extracted with EtOAc (3 x 30 ml), dried, evaporated, the resulting residue (0.862 g) digested with satd. aq. $NaHCO_3$ (40 ml), extracted with EtOAc (3 x 30 ml), dried, evaporated and chromatographed over silica gel. Elution with PhH : EtOAc :: 3:7 gave 0.351 g (22%) of ZAsn(β-$CONH_2$)-OMe, (36), mp. 168-169°C.

ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1740 (ester), 1690, 1660, 1530 (amide).

nmr : δ($CDCl_3$ + DMSO-d_6) : 2.8 (d, 2H, -CHC$\underline{H}_2$), 3.65 (s, 3H, -COOC$\underline{H}_3$), 4.3-4.7 (m, 1H, -C$\underline{H}$), 5.05 (s, 2H, -OC$\underline{H}_2$Ph), 6.7-7.1 (br, 2H,-CO-N$\underline{H}_2$), 7.3 (s, 5H, aromatic protons), 7.8-8.1 (m, 1H, -CHN$\underline{H}$), 10.1 (s, 1H, -CON$\underline{H}$CONH$_2$).

ms : m/z : 323 (M^+), 324 (M^++1).

anal : Found : C, 52.23; H, 5.14; N, 12.89%

Calc. for $C_{14}H_{17}O_6N_3$: C, 52.01; H, 5.26; N, 13.00%

The bicarbonate extract was adjusted to pH~3 with cold 2N H_2SO_4, extracted with EtOAc (3 x 30 ml), dried, evaporated and the resulting crude acid on treatment with CH_2N_2 followed by chromatography over silica gel and elution with PhH : EtOAc :: 7:3, afforded 0.361 g (25%) of ZAsp(β-OMe)OMe, (17), identical in all respects with that of an authentic sample.

XLVI. Reaction of N-Benzoyl L-Methionine Methyl Ester (BzMetOMe, 8) with

Ru^{VIII}: Isolation of N-Benzoyl Methionine Sulfone Methyl Ester ($BzMet(SO_2)$-OMe, 37) and N-Benzoyl Methionine Sulfone ($BzMet(SO_2)OH$, 38) :

A mixture of BzMetOMe, (8), (1.5 g, 5.61 mmol), MeCN (22.4 ml), CCl_4 (22.4 ml), H_2O (44.8 ml), $NaIO_4$ (21.599 g, 101 mmol) and $RuCl_3.3H_2O$ (0.042 g, 2.2 mol%) was sealed, left shaken for 60 h and separated into neutral and acidic fractions as described previously.

The neutral fraction on crystallization from dry EtOAc-Hexane gave 0.11 g (7%) of $BzMet(SO_2)OMe$, (37), mp. 118-119°C (lit.[95] mp. 106°C).

ir : ν_{max}(KBr) cm^{-1} : 3350 (-NH), 1760 (-CO), 1660, 1550 (amide), 1310, 1255, 1145 ($-SO_2$).

nmr : δ ($CDCl_3$) : 2.9 (s, 3H, $-SO_2CH_3$), 3.0-3.4 (m, 4H, $-CH(CH_2)_2$), 3.75 (s, 3H, $-COOCH_3$), 4.9 (q, 1H, -CH), 7.1-7.9 (m, 6H, -NH, aromatic protons).

ms : m/z : 299 (M^+).

The acidic residue, 1.075 g (65%) was identified as $BzMet(SO_2)OH$, (38), via transformation to the corresponding methyl ester (37) with CH_2N_2, chromatography over silica gel, elution with PhH : EtOAc :: 6:4 and crystallization from dry EtOAc-Hexane to yield $BzMet(SO_2)OMe$, (37), mp. 118-119°C, identical in all respects to that obtained from the neutral fraction (vide supra).

XLVII. Reaction of N-Benzyloxycarbonyl L-Methionine Methyl Ester (ZMetOMe, 9) with Ru^{VIII}: Isolation of N-Benzyloxycarbonyl Methionine Sulfone Methyl Ester ($ZMet(SO_2)OMe$, 39) and N-Methyloxycarbonyl Methionine Sulfone

Methyl Ester (MeOCOMet(SO_2)OMe, 40) :

A mixture of ZMetOMe, (9), (1.5 g, 5.05 mmol), MeCN (20.2 ml), CCl_4 (20.2 ml), H_2O (40.4 ml), $NaIO_4$ (19.446 g, 90.9 mmol) and $RuCl_3.3H_2O$ (0.038 g, 2.2 mol%) was sealed, left shaken for 60 h at rt and separated into neutral and acidic fractions as described previously.

The neutral fraction on crystallization from dry EtOAc-Hexane gave 0.401 g (40%) of ZMet(SO_2)OMe (39), mp. 89°C (lit.[95] mp. 89°C).

ir : ν_{max}(KBr) cm^{-1} : 3320 (-NH), 1730 (ester), 1690, 1530, 1320, 1270, 1125 (SO_2).

nmr : $\delta(CDCl_3)$: 2.0-2.55 (m, 2H, -CHC<u>H</u>$_2$), 2.8 (s, 3H, -SO_2C<u>H</u>$_3$), 2.9-3.3 (m, 2H, -C<u>H</u>$_2$$SO_2CH_3$), 3.7 (s, 3H, -COOC<u>H</u>$_3$), 4.1-4.65 (m, 1H, -C<u>H</u>), 5.05 (s, 2H, -OC<u>H</u>$_2$Ph), 5.35-5.9 (br, 1H, -N<u>H</u>), 7.25 (s, 5H, aromatic protons).

ms : m/z : 329 (M^+), 330 (M^++1).

The acidic fraction was esterified with CH_2N_2 and the residue chromatographed over silica gel. Elution with PhH : EtOAc :: 3:7 gave 0.230 g (18%) of MeOCOMet(SO_2)OMe, (40), as a thick syrup.

ir : ν_{max}(neat) cm^{-1} : 3310 (-NH), 1725, 1520, 1440.

nmr : δ ($CDCl_3$ + DMSO-d_6) : 2.05 - 2.65 (br, 2H, -CHC<u>H</u>$_2$), 2.9 (s, 3H, -SO_2C<u>H</u>$_3$), 3.15 (t, 2H, -C<u>H</u>$_2$$SO_2CH_3$), 3.65 (s, 6H, 2 x -$CO_2$C<u>H</u>$_3$), 4.7-5.0 (br, 1H, -C<u>H</u>).

ms : m/z : 253 (M^+).

anal : Found : N, 5.80%

Calc. for $C_8H_{15}O_6NS$: C, 37.94; H, 5.93; N, 5.53%

XLVIII. Reaction of N-Benzyloxycarbonyl S-Benzyl L-Cysteine Methyl Ester (ZCys(S-Bzl)OMe, 10) with Ru^{VIII} : Isolation of N-Benzyloxycarbonyl Cysteine S-Benzyl Sulfone Methyl Ester (ZCys(SO_2-Bzl)OMe, 41) :

A mixture of ZCys(S-Bzl)OMe, (10), (0.718 g, 2 mmol), MeCN (8 ml), CCl_4(8 ml), H_2O (16 ml), $NaIO_4$ (7.7 g, 36 mmol) and $RuCl_3.3H_2O$ (0.015 g, 2.2 mol%) was sealed, left shaken for 60 h at rt and worked up as described previously. The neutral portion yielded 0.233 g (30%) of ZCys(SO_2-Bzl)OMe, (41), mp. 174-175°C (dry EtOAc-Hexane) (lit.[95] mp. 174°C).

ir : ν_{max}(KBr) cm^{-1} : 3315 (-NH), 1730 (ester), 1685, 1520, 1300, 1250, 1130, (-SO_2).

nmr : $\delta(CDCl_3)$: 3.5 (d, 2H, -CHC$\underline{H}_2$), 3.75 (s, 3H, -COOC$\underline{H}_3$), 4.2 (s, 2H, -SO_2C$\underline{H}_2$Ph), 4.65-4.85 (br, 1H, -C$\underline{H}$), 5.1 (s, 2H, -OC$\underline{H}_2$Ph), 5.85 (br, 1H, -N$\underline{H}$), 7.2-7.4 (m, 10H, aromatic protons).

ms : m/z : 391 (M^+).

XLIX. Reaction of N-Benzoyl L-Proline Methyl Ester (BzProOMe, 11) with Ru^{VIII}: Isolation of N-Benzoyl Pyroglutamic Acid Methyl Ester (Bz-pyroGlu-OMe, 42) and N-Benzoyl L-Glutamic Acid α-Methyl Ester (BzGlu(γ -OH)-OMe, 43) :

A mixture of BzProOMe, (11), (1.165 g, 5 mmol), MeCN (20 ml), CCl_4(20 ml), H_2O (40 ml), $NaIO_4$(19.25 g, 90 mmol) and $RuCl_3.3H_2O$ (0.038g,

2.2 mol%) was left stirred at rt for 60 h, filtered, the residue washed with CCl_4 (3 x 15 ml), the aqueous layer extracted with EtOAc (3 x 30 ml), the combined organic extracts dried, evaporated, the residue digested with satd. aq. $NaHCO_3$, extracted with EtOAc (3 x 30 ml), dried and evaporated to yield 0.460 g (40%) of product identified as Bz-pyroGluOMe, (42), mp. 154°C (dry PhH-Hexane).

ir : ν_{max}(KBr) cm^{-1} : 3460, 3300, 1740 (ester), 1660, 1595.

nmr : $\delta(CDCl_3)$: 1.8-2.9 (m, 4H, $-CH(C\underline{H}_2)_2$), 3.75 (s, 3H, $-COOC\underline{H}_3$), 4.82 (br, 1H, $-C\underline{H}$), 6.9-8.0 (m, 5H, aromatic protons).

ms : m/z : 247 (M^+), 248 (M^++1).

The bicarbonate extracted was adjusted to pH~3 with 2N H_2SO_4, saturated with NaCl, extracted with EtOAc (3 x 30 ml), dried, evaporated and esterified with ethereal CH_2N_2 to yield 0.240 g (17%) of BzGlu(γ-OMe)OMe, whose properties were identical with that of an authentic sample, (20), Experiment XVIII.

L. Reaction of N-Benzoyl L-Serine Methyl Ester (BzSerOMe, 12) with Ru^{VIII} at pH 6 : Isolation of Benzamide :

A solution of BzSerOMe (0.446 g, 2 mmol) in MeCN (8 ml) was admixed with CCl_4 : H_2O :: 1:2 (24 ml), $NaIO_4$ (7.7 g, 36 mmol), $RuCl_3.3H_2O$ (0.015 g, 2.2 mol%), stirred for 60 h at rt and worked up precisely as described in previous experiments to give 0.190 g of residue. Trituration for 3 h with satd. aq. $NaHCO_3$ (20 ml), extraction with EtOAc (3 x 15 ml), drying and evaporation of the EtOAc extract afforded compound, mp. 125°C (dry EtOAc-Hexane) which was identified as benzamide; yield 0.141 g (58.3%).

The bicarbonate extract on work up afforded small amounts (0.044 g) of intractable residue.

LI. Reaction of N-Benzoyl L-Serine Methyl Ester (BzSerOMe, 12) with Ru^{VIII} at pH 3 : Demonstration of the control of pH on reactivity :

A solution of BzSerOMe (0.446 g, 2 mmol) in MeCN (8 ml) was admixed with CCl_4 : Phosphate buffer (pH 3) :: 1:2 (24 ml), $NaIO_4$ (7.7 g, 36 mmol), $RuCl_3.3H_2O$ (0.015 g, 2.2 mol%), stirred for 72 h at rt and worked up precisely as described in previous experiments to afford 0.340 g of unchanged starting material as the neutral residue.

A careful analysis revealed the absence of any other pure product in the reaction mixture.

LII. Reaction of N-Benzoyl L-Threonine Methyl Ester (BzThrOMe, 13) with Ru^{VIII} at pH 6 : Isolation of Benzamide :

A solution of BzThrOMe (0.95 g, 4 mmol) in MeCN (16 ml) was admixed with CCl_4: H_2O :: 1:2 (48 ml), $NaIO_4$(15.4 g, 72 mmol), $RuCl_3.3H_2O$ (0.030 g, 2.2 mol%), sealed, left shaken for 60 h at rt, cautiously opened and worked upto give 0.220 g of residue which, when further processed, gave 0.121 g (25%) of benzamide, mp. 125-126°C (dry EtOAc-Hexane).

The bicarbonate extract failed to yield any pure product.

LIII. Reaction of N-Benzoyl L-Threonine Methyl Ester (BzThrOMe, 13) with Ru^{VIII} at pH 3 : Isolation of Benzamide :

A solution of BzThrOMe (0.474 g, 2 mmol) in MeCN (8 ml) was

admixed with CCl_4: Phosphate buffer (pH 3) :: 1:2 (24 ml), $NaIO_4$ (7.7 g, 36 mmol), $RuCl_3.3H_2O$ (0.015 g, 2.2 mol%), stirred for 18 h at rt and worked up precisely as described in previous experiments to yield, as the neutral fraction, 0.203 g (84%) of benzamide, mp. 124°C.

No other product could be isolated from this experiment.

LIV. Reaction of N-Benzyloxycarbonyl L-Asparagine Methyl Ester (ZAsnOMe, 15) with Ru^{VIII} :

A mixture of ZAsnOMe, (15),[96] (0.450 g, 1.6 mmol), MeCN (6.4 ml), CCl_4: H_2O :: 1:2 (19.2 ml), $NaIO_4$ (6.160 g, 28.8 mmol) and $RuCl_3.3H_2O$ (0.012 g, 2.2 mol%) was mechanically stirred for 60 h at rt. The reaction mixture was filtered and worked up exactly as described before to yield 0.172 g of intractable residue. No pure compounds could be isolated from this reaction.

LV. Reaction of N-Benzyloxycarbonyl L-Glutamine Methyl Ester (ZGlnOMe, 16) with Ru^{VIII} at pH 6 : Demonstration of the lack of reactivity of the Glutamine side chain at pH 6 :

A mixture of ZGlnOMe, (16),[97] (1 g, 3.4 mmol), MeCN (13.6 ml), CCl_4: H_2O :: 1:2 (40.8 ml), $NaIO_4$ (13.09 g, 61.2 mmol) and $RuCl_3.3H_2O$ (0.026 g, 2.2 mol%) was vigorously stirred for 60 h at rt. Work up exactly as outlined before only led to the recovery of 0.673 g of unreacted starting material. No other products could be isolated from the reaction mixture.

LVI. Reaction of N-Benzyloxycarbonyl L-Glutamine Methyl Ester (ZGlnOMe, 16) with Ru^{VIII} at pH 3 : Demonstration of the lack of reactivity of the

Glutamine side chain at pH 3 :

A solution of ZGlnOMe, (16), (0.588 g, 2 mmol) in MeCN (8 ml) was admixed with CCl_4: Phosphate buffer (pH 3) :: 1:2 (24 ml), $NaIO_4$ (7.70 g, 36 mmol), $RuCl_3.3H_2O$ (0.015 g, 2.2 mol%), mechanically stirred for 18 h at rt, the pH of the reaction brought to ~6 with satd. aq. $NaHCO_3$, filtered, the residue washed with CCl_4(3 x 10 ml) and the filterate worked up as described before to yield 0.365 g of unreacted started material. No other compounds could be isolated from the reaction mixture.

LVII. Reaction of N^{α}-benzoyl L-Arginine Ethyl Ester Hydrochloride (BzArg-OEt.HCl) with Ru^{VIII} at pH 6 : Demonstration of the inertness of the Arginine side chain at pH 6 :

A mixture of BzArgOEt.HCl (0.172 g, 0.5 mmol), MeCN (2 ml), CCl_4: H_2O :: 1:2 (6 ml), $NaIO_4$ (1.925 g, 9 mmol) and $RuCl_3.3H_2O$ (0.004 g, 2.2 mol%) was stirred for 100 h at rt. The reaction mixture was filtered, the residue washed with cold MeOH (3 x 10 ml) and the combined filterates evaporated to dryness. The thoroughly dried residue was triturated with ice-cooled dry MeOH (5 x 5 ml) and the MeOH extract evaporated to yield 0.071 g of unchanged starting material. No other products were isolated from this reaction.

LVIII. Reaction of N^{α}-Benzoyl L-Arginine Ethyl Ester Hydrochloride (BzArg-OEt.HCl) with Ru^{VIII} at pH 3 : Demonstration of the inertness of the Arginine side chain at pH 3 :

A mixture of BzArgOEt.HCl (0.172 g, 0.5 mmol), MeCN (2 ml), CCl_4: Phosphate buffer (pH 3) :: 1:2 (6 ml), $NaIO_4$ (1.925 g, 9 mmol) and

$RuCl_3.3H_2O$ (0.004g, 2.2 mol%) was stirred for 60 h at rt. The reaction mixture was worked up as described in Experiment LVI, the combined filterates evaporated, the residue thoroughly dried, triturated with ice-cooled dry MeOH (5 x 5 ml) and the MeOH extract evaporated to dryness to yield 0.137 g of residue, which, on elution as 0.1 N solution through a column of IRA 400 Cl^- (regenerated using 0.1 N HCl), gave 0.093 g of unreacted starting material. Work up and careful analysis of the reaction mixture did not yield any other product.

LIX. Reaction of N^{α}-Benzyloxycarbonyl L-Lysine (N^{α}-Z Lys-OH, 14) with RuO_4 at pH 3 : Demonstration of the control of pH on the reactivity of the Lysine side chain :

A mixture of N^{α}-Z Lys-OH, (14), (0.100 g, 0.35 mmol), MeCN (1.5 ml), CCl_4: Phosphate buffer (pH 3) :: 1:2 (4.5 ml), $NaIO_4$ (1.374 g, 6.275 mmol) and $RuCl_3.3H_2O$ (0.003 g, 2.2 mol%) were stirred at rt for 18 h and worked up as described in Experiment LVI. Evaporation of all solvents and trituration of the thoroughly dried residue with ice-cooled dry MeOH (3 x 10 ml) and then hot, dry MeOH (3 x 10 ml) followed by pooling and evaporation of the MeOH extracts yielded 0.072 g of unreacted starting material. No other products could be isolated from the reaction mixture.

LX. Reaction of N-^{t}Butyloxycarbonyl L-Phenylalanyl L-Phenylalanine Methyl Ester (BocPhe-PheOMe, 21) with Ru^{VIII} with restricted amount of periodate :

The reaction of BocPhe-PheOMe, (21), (0.3 g, 0.7 mmol) in MeCN-CCl_4-H_2O using 2.8 mmol of $NaIO_4$ and 2.2 mol% of $RuCl_3.3H_2O$ for 60 h followed by work up led to the recovery of the starting material.

LXI. Reaction of N-Benzoyl L-Tyrosyl L-Phenylalanine Methyl Ester (BzTyr-PheOMe, 22) with Ru^{VIII} : Isolation of N-Benzoyl L-Aspartyl L-Aspartic Acid α -Methyl Ester (BzAsp(β -OH)-Asp(β -OH)OMe, 44) :

A mixture of BzTyr-PheOMe,(22), (0.892 g, 2 mmol), MeCN : CCl_4: H_2O :: 8:8:16 ml, $NaIO_4$ (7.7 g, 36 mmol) and $RuCl_3.3H_2O$ (0.015 g, 2.2 mol%) was sealed, left shaken for 12 h at rt and worked up as described previously.

The reaction afforded negligible amounts of neutral residue and 0.58 g of acidic mixture which was identified as BzAsp(β -OH)-Asp(β -OH)OMe, (44), (73.6%) via conversion with ethereal CH_2N_2 to BzAsp(β -OMe)-Asp(β -OMe)-OMe,(27), and comparison with an authentic sample.

LXII. Reaction of N-Benzoyl L-Tryptophyl L-Leucine Methyl Ester (BzTrp-LeuOMe, 23) with Ru^{VIII}: Isolation of N-Benzoyl L-Aspartyl L-Leucine Methyl Ester (BzAsp-LeuOMe, 45) :

A mixture of BzTrp-LeuOMe,(23), (1.0 g, 2.3 mmol), MeCN : CCl_4: H_2O (10:10:20 ml), $NaIO_4$ (8.84 g, 41.4 mmol) and $RuCl_3.3H_2O$ (0.017 g, 2.2 mol%) was sealed, left shaken for 60 h, filtered, washed with CCl_4, the aqueous layer extracted with EtOAc (3 x 20 ml), the combined organic extracts dried, evaporated, the residue digested with satd. aq. $NaHCO_3$ (25 ml) for 3 h, extracted with EtOAc to remove small amounts of neutral material, adjusted to pH~ 3 with cold 2N H_2SO_4, saturated with NaCl, extracted with EtOAc (3 x 30 ml), washed with satd. NaCl, dried and evaporated to give 0.52 g (58%) of BzAsp-LeuOMe,(45), whose structure was further confirmed by esterification to BzAsp-(β -OMe)-LeuOMe,(28), and comparison with an authentic sample.

LXIII. Reaction of N-Benzoyl L-Tryptophyl L-Phenylalanine Methyl Ester (BzTrp-PheOMe, 24) with Ru^{VIII}: Demonstration of the preferential oxidation of the Tryptophan residue under controlled conditions : Isolation of N^{α}-Benzoyl N^{ω}-Formylkynurenyl L-Phenylalanine Methyl Ester (N^{α}-Bz N^{ω}-ForKyn-PheOMe, 46) and N-Benzoyl L-Aspartyl L-Phenylalanine Methyl Ester (BzAsp-PheOMe, 47) :

A mixture of BzTrp-PheOMe, (24), (1.2 g, 2.13 mmol), MeCN : CCl_4: H_2O (8.5 : 8.5 : 17 ml), $NaIO_4$ (8.2 g, 38.34 mmol) and $RuCl_3.3H_2O$ (0.016 g, 2.2 mol%) was sealed, left shaken for a very restricted duration of 8 h at rt and worked up precisely as described in the previous experiment.

The neutral fraction gave 0.14 g (13%) of N^{α}-Bz N^{ω}-ForKyn-PheOMe, (46), mp. 181-183°C (dry PhH-Hexane).

ir : ν_{max}(KBr) cm^{-1} : 3260 (-NH), 1730 (ester), 1630, 1520 (amide).

nmr : $\delta(CDCl_3)$: 3.1 (d, 2H, -CHC$\underline{H}_2$Ph), 3.7 (m, 5H, -CHC$\underline{H}_2$CO, -COOC$\underline{H}_3$), 4.8 (br, 1H, -C$\underline{H}$), 5.2 (br, 1H, -C$\underline{H}$), 6.8-7.9 (m, 15H, -N$\underline{H}$, aromatic protons), 8.3 - 8.8 (br, 2H, PhCON$\underline{H}$, -N$\underline{H}$CHO).

ms : m/z : 501 (M^+), 502 (M^++1).

anal : Found : C, 67.16; H, 5.32; N, 8.29%

Calc. for $C_{28}H_{27}O_6N_3$: C, 67.07; H, 5.39; N, 8.38%

$[\alpha]_D^{25}$: +72.82 (c, 0.46; $CHCl_3$).

The bicarbonate extract, when processed, gave 0.6 g (66%) of BzAsp-PheOMe, (47), whose structure was further confirmed by transformation with

CH_2N_2 to BzAsp(β -OMe)-PheOMe,(29), found to be identical with an authentic sample (Experiment XXVI).

LXIV. Reaction of N-Benzoyl L-Prolyl L-Phenylalanine Methyl Ester (BzPro-PheOMe, 25) with Ru^{VIII} : Isolation of N-Benzoyl L-Glutamyl L-Aspartic Acid Methyl Ester (BzGlu-AspOMe, 48) and N-Benzoyl L-Prolyl L-Aspartic Acid Methyl Ester (BzPro-AspOMe, 49) :

A mixture of BzPro-PheOMe,(25), (0.76 g, 2 mmol), MeCN : CCl_4 : H_2O (8 : 8 : 16 ml), $NaIO_4$ (7.7 g, 36 mmol) and $RuCl_3.3H_2O$ (0.015 g, 2.2 mol%) was sealed and left shaken for a restricted time duration of 12 h at rt. Work up, as described previously, gave, from the neutral fraction, 0.15 g of starting material.

The bicarbonate extract afforded 0.348 g of residue which on treatment with CH_2N_2 followed by chromatography of the resulting esters over silica gel and elution with PhH : EtOAc :: 4:6, gave, 0.087 g (14%) of BzGlu(γ -OMe)-Asp(β -OMe)OMe, (31), identical in all respects with an authentic sample (Experiment XXVIII).

Further elution with PhH : EtOAc :: 3 : 7 gave 0.169 g (29%) of BzPro-Asp(β -OMe)OMe, (30), which was found to be identical with an authentic sample (Experiment XXVII).

LXV. Reaction of N-Benzoyl L-Phenylalanyl L-Proline Methyl Ester (BzPhe-ProOMe, 26) with Ru^{VIII} : The exclusive formation of N-Benzoyl L-Aspartyl L-Proline Methyl Ester (BzAsp-ProOMe, 50) :

A mixture of BzPhe-ProOMe,(26), (0.7 g, 1.84 mmol), MeCN : CCl_4: H_2O :: 7.4 : 7.4 : 14.8 ml, $NaIO_4$ (7.092 g, 33.2 mmol) and $RuCl_3.3H_2O$ (0.014 g,

2.2 mol%) was sealed, left shaken for 8 h at rt and worked up precisely as described in previous experiments. The neutral fraction yielded 0.137 g of unreacted starting material.

The bicarbonate extract was adjusted to pH~3 with cold 2N H_2SO_4, extracted with EtOAc (3 x 25 ml), washed with satd. NaCl (30 ml), dried, evaporated and the resulting residue directly converted to the methyl ester using ethereal diazomethane. The crude esterified product, on chromatography over silica gel followed by elution with PhH : EtOAc :: 1:1, afforded 0.331 g (62%) of product which was identified as BzAsp(β-OMe)-ProOMe, obtained as a gummy solid.

ir : ν_{max}(neat) cm^{-1} : 3300 (-NH), 1725 (ester), 1635, 1525 (amide).

nmr : $\delta(CDCl_3)$: 1.5-2.45 (m, 4H, $-CH(C\underline{H}_2)_2$), 2.75 (d, 2H, $-CHC\underline{H}_2Ph$), 3.1-4.1 (m,8H, 2 x $-COOC\underline{H}_3$, $-(CH_2)_2$ $C\underline{H}_2N$), 4.35 (m, 1H, $-C\underline{H}$), 5.3 (m, 1H, $-C\underline{H}$), 6.5 (br, 1H, $-N\underline{H}$), 7.0-8.1 (m, 5H, aromatic protons).

ms : m/z : 362 (M^+), 363 (M^++1).

LXVI. Reaction of N-Benzyloxycarbonyl L-Leucyl L-Prolyl L-Leucyl L-Tryptophan Methyl Ester (ZLeu-Pro-Leu-TrpOMe, 51) with Ru^{VIII}: Isolation of N-Benzyloxycarbonyl L-Leucyl L-Prolyl L-Leucyl L-Aspartic Acid α-Methyl Ester (ZLeu-Pro-Leu-AspOMe, 52) :

A mixture of ZLeu-Pro-Leu-TrpOMe, (51), (0.030 g, 0.44 mmol), MeCN : CCl_4: H_2O :: 0.4 : 0.4 : 0.8 ml, $NaIO_4$ (0.169 g, 7.92 mmol), and $RuCl_3.3H_2O$ (1 mg) was sealed, left shaken for 8 h at rt, filtered, the residue washed with EtOAc (5 x 5 ml), the combined organic and aqueous filterates evaporated to dryness under vacuo, extracted with EtOAc (5 x 5 ml) followed by MeOH -

(3 x 5 ml), the combined extracts evaporated and dried to give 0.032 g of residue. Amino acid analysis of this showed the clean transformation of tryptophan to aspartic acid and on the basis of this analysis, the (51)→(52) conversion was estimated to have occurred in 60% yield.

LXVII. Reaction of BocLeu-Val-Leu-Phe-Leu-Pro-Leu-Ala-Ala-Leu-GlyOBzl (53) with Ru^{VIII}: Isolation of BocLeu-Val-Leu-Asp-Leu-Pro-Leu-Ala-Ala-Leu-GlyOBzl (54) :

A mixture of (53), (0.1 g, 0.0759 mmol), MeCN : CCl_4 : H_2O :: 2 : 2 : 4 ml, $NaIO_4$ (0.584 g, 2.732 mmol) and $RuCl_3.3H_2O$ (1 mg) was sealed, left shaken for 60 h at rt and worked up precisely as described in Experiment LXVI relating to the transformation of the tetrapeptide (51). Amino acid analysis of the crude reaction product clearly demonstrated that the required Phe→Asp change had taken place in quantitative yields and that the observed experimental results with regard to having the Pro residue being unaffected by this reaction was as anticipated.

LXVIII. The Chemoselective Transformation of Melittin (Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-$GlnNH_2$, 55) to Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Asp-Ile-Lys-Arg-Lys-Arg-Gln-$GlnNH_2$ (56) with Ru^{VIII} :

A mixture of Melittin (0.002 g, 0.0007 mmol), MeCN : CCl_4: Phosphate buffer (pH 3) :: 0.1 : 0.1 : 0.2 ml, $NaIO_4$ (0.0027 g, 18 eq), and $RuCl_3.3H_2O$ (0.5 mg) was sealed, left shaken for 8 h at rt, passed through a small wad of

cotton wool, washed with CCi_4 and MeCN, evaporated, the residue extracted with MeOH (10 x 0.5 ml), the MeOH extract evaporated and the resulting product (0.003 g) was subjected to amino acid and spectral analyses. The spectral data indicated the complete absence of any aromatic moiety in the product. The ORD spectrum of the crude product was identical to that of the parent. Amino acid analysis was in expectation to that anticipated excepting for the residues Pro and Arg, specialized analyses and identification of which could not be carried out because of paucity of material.

LXIX. The reactions of Tyrosine, Phenylalanine and Lysine with Ru^{VIII} under different pH conditions :

These series of experiments are presented in a tabular form below :

TABLE[a]

Substrate	Conditions	Product (% Yield)
p $OH-C_6H_4CH_2CH(NH_2)CO_2H$ (Tyrosine)	pH 3[b] , 50 min	$HO_2C-CH_2CH(NH_2)CO_2H$ (50)
,,	pH 6[c] , 3 h	$HO_2C-CH_2-CO_2H$ (20)
,,	pH 9[d], 3 h	$HO_2C-CH_2-CO_2H$ (48)
$PhCH_2CH(NH_2)CO_2H$ (Phenylalanine)	pH 3, 50 min	$PhCH_2CO_2H$ (76)
,,	pH 6, 3 h	$PhCH_2CO_2H$ (43)
$H_2N-(CH_2)_4CH(NH_2)CO_2H$ (Lysine)	pH 3, 50 min	$H_2N-CO-(CH_2)_3CO_2H$ (34)
,,	pH 6, 3 h	$H_2N-CO-(CH_2)_3CO_2H$ (33)

-- contd.

a : MeCN : CCl_4 : Phosphate buffer (pH 3)[b]/ H_2O[c]/ satd. aq. $NaHCO_3$[d] :: 16 : 16 : 32 :ml/ 4 mmol of substrate, $NaIO_4$ (72 mmol), $RuCl_3.3H_2O$ (2.2 mol%) stirred at rt. After the reaction, the pH was adjusted to ~ 6 in every case and extracted with EtOAc. Malonic acid, was obtained from the organic phase.

In the case of phenylacetic acid and glutaric acid monoamide, in addition to the first crop obtained from direct EtOAc extract, the aqueous layer was evaporated to dryness and extracted with EtOAc (5 x 15 ml) to yield a second crop. In the case where aspartic acid was isolated, the aqueous layer, after NaCl saturation, was continuously extracted with Et_2O for 4 days. This was done in every experiment involving tyrosine.

Lysine, both at pH 3 and at pH 6, gave glutaric acid monoamide as the exclusive product; mp. 102°C.

ir : ν_{max}(KBr) cm^{-1} : 3310 (-NH), 1685 (acid), 1620, 1520 (amide).

nmr : $\delta(CDCl_3)$: 1.8 (m, 2H, $-CH_2\underline{CH_2}CH_2$), 2.3 (m, 2H, $-CH_2\underline{CH_2}CONH_2$), 3.25 (m, 2H, $-\underline{CH_2}CO_2H$), 7.1-8.7 (br, 2H, $-CO\underline{NH_2}$).

anal : Found : C, 45.66; H, 7.20; N, 10.34%

Calc. for $C_5H_9O_3N$: C, 45.8; H, 6.87; N, 10.69%

Glutaric acid monoamide was further characterized by transformation using ice-cooled ethereal CH_2N_2 to it's methyl ester which was obtained as an oil.

nmr : $\delta(CDCl_3)$: 1.9 (m, 2H, $-CH_2\underline{CH_2}CH_2$), 2.35 (t, 2H, $-\underline{CH_2}CONH_2$), 3.3(m,2H, $-\underline{CH_2}CO_2CH_3$), 3.65(s,3H, $-COO\underline{CH_3}$), 8.1(br s,2H, $-CO\underline{NH_2}$).

: m/z : 145 (M^+).

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VITAE

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